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PART 1
SURVEY OF PHYSIOLOGICAL MECHANISMS OF SODIUM AND CHLORIDE ION TRANSPORT AND DESIGN OF EXPERIMENT FOR APPLICATION TO DEMINERALIZING SALINE WATERS

PART 2
LABORATORY EVALUATION OF USE OF ALGAE IN SALINE WATER CONVERSION
– PHASE I FINAL REPORT –

By

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Washington, D. C.

For
OFFICE OF SALINE WATER
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FOREWORD

This is the fifty-second of a series of reports designed to present accounts of progress in saline water conversion with the expectation that the exchange of such data will contribute to the long-range development of economical processes applicable to large-scale, low-cost demineralization of sea or other saline water.

Except for minor editing, the data herein are as contained in the reports submitted by Resources Research Incorporated under Contract No. 14-01-001-96, covering research carried out through October 15, 1957, and Contract No. 14-01-001-109, covering research through November 30, 1958. The data and conclusions given in this report are essentially those of the Contractor and are not necessarily endorsed by the Department of the Interior.

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Part 1

SURVEY OF PHYSIOLOGICAL MECHANISMS OF SODIUM AND CHLORIDE ION TRANSPORT AND DESIGN OF EXPERIMENT FOR APPLICATION TO DEMINERALIZING SALINE WATERS

I. Summary

All living organisms constantly accumulate and excrete salts, including sodium chloride, from aqueous solutions. The processes by which this is accomplished, however, have received little, if any, scrutiny by persons primarily interested in the reclamation of saline waters. In the belief that a review of the scientific knowledge on the subject might reveal possible engineering applications, a literature survey was made. The medical, biochemical and physiological literature was searched for information regarding natural processes by which salt concentration gradients are established and maintained between organisms and their environment.

The search was begun by examining information relating to the function of the human kidney which conserves electrolytes within the body by absorbing them from the urine. The salt-conserving actions of the sweat and secretory glands were also examined. Kidney and other salt-concentrating functions in animals were reviewed. Concentration of sodium chloride is effected by intestinal mucosa, gills of fish and the skin of amphibians. Plants, single-celled organisms and tissue cells were also studied in regard to the accumulation and excretion of salts.

Knowledge concerning how living organisms accumulate or excrete salts is very limited. Scientific investigation in this area is complex and difficult. However, on the basis of available information, there seems to be a striking similarity in physiological mechanisms achieving electrolyte balance. Factors in common with many varieties of animals, plants and cells are the abilities to accumulate and maintain ions against considerable

concentration gradients through the expenditure of energy derived from metabolic processes. This ability is termed "active transport."

Many theories and variations have been offered by investigators in attempts to explain the active transport of ions. Some of the proposed theories indulge in intricate mathematical treatments probably not warranted by the scant facts available. The more prominent theories, on the basis of acceptance in the field, are reviewed in this report.

Many investigators have studied sodium chloride transport through the isolated frog skin. Calculations based on these data show that very large surface areas are required for appreciable transport of sodium chloride. Calculations also indicate, however, that the energy requirements closely approach the theoretical minimum for the separation of salt and water by any possible means. Because of the similarity of sodium chloride transport in living organisms, these calculations may provide a very rough approximation of the area-energy relationship for physiological transport other than in frog skin.

A method seeking to utilize physiological transport of sodium chloride by living organisms for application to the conversion of saline waters is proposed. This envisions the use of unicellular algae in a sodium chloride "conveyor belt" process. Grown in an optimum environment of saline water, the algae, possessing large surface area per unit volume, would accumulate sodium chloride. They would then be mechanically removed to an unfavorable environment where the source of metabolic energy necessary to maintain the internal concentration of sodium chloride would be denied.

This might be accomplished by excluding light. The algae should consequently dump sodium chloride in approaching equilibrium with the environment. After this, they would be removed from the enriched suspension and returned to the original suspension where they would accumulate more sodium chloride, gradually depleting the salinity of the water as the cycling continues. A flow diagram of the hypothesized process is presented and explained. The prospects of the method are believed to warrant a laboratory investigation.

II. Introduction

One of the fundamental life processes is the ability of plants and animals to transport and accumulate or excrete selectively many types of ions. Among these ions are sodium and chloride. A great amount of basic and applied research has been conducted in the field of ion transport by physiological mechanisms. Very little, if any, of this work, however, has been concerned with sodium and chloride with a view toward possible significance in saline water recovery. This study was undertaken to explore the physiological processes for possible practical applications to the extraction of fresh water from sea water or brackish water.

The study had two principal objectives which also indicate the manner in which it was conducted:

1. To survey the medical, physiological and biochemical knowledge relating to the separation of sodium and chloride ions from water, and

2. To employ the above disciplines in conjunction with those of engineering in designing an experiment for developing a practical method of utilizing natural principles in low-energy removal of sodium and chloride ions from saline waters.

In the early stages of the study, considerable emphasis was placed on kidney function since the kidney is an outstanding example of an organ which can separate sodium and chloride ions from aqueous solution. It routinely does this in the production of urine. It was soon learned that, while the kidney performs as an integrated organ, the actual secretion or reabsorption of ions is accomplished at the cellular or even subcellular level. This ability is not peculiar to cells of kidney tissue, but is common to all living cells. Selectivity of the cell membrane to the

passage of ions and the utilization of energy derived from metabolism to establish and maintain concentration gradients across the membrane are the common denominators. Accordingly, the search was broadened to include areas of animal and plant physiology.

Limitations of the study made a complete survey of the vast literature on the subjects impossible. With the assistance of expert guidance, the more significant and representative material was selected. In this manner, the important observations and theories relevant to the objects of the study have been reviewed.

The input energies and efficiencies of physiological processes seem to offer advantages over mechanical methods of separating salt and water. As yet the biochemistry of these processes is obscure. In addition, physiological transport depends upon detailed and complex physiological structure which cannot be reproduced artificially. However, a possibility of applying physiological principles directly through the utilization of living organisms may exist. A description of an hypothesized process which would induce algae to behave as a sodium chloride "conveyor belt" is presented.

III. Macro Organisms

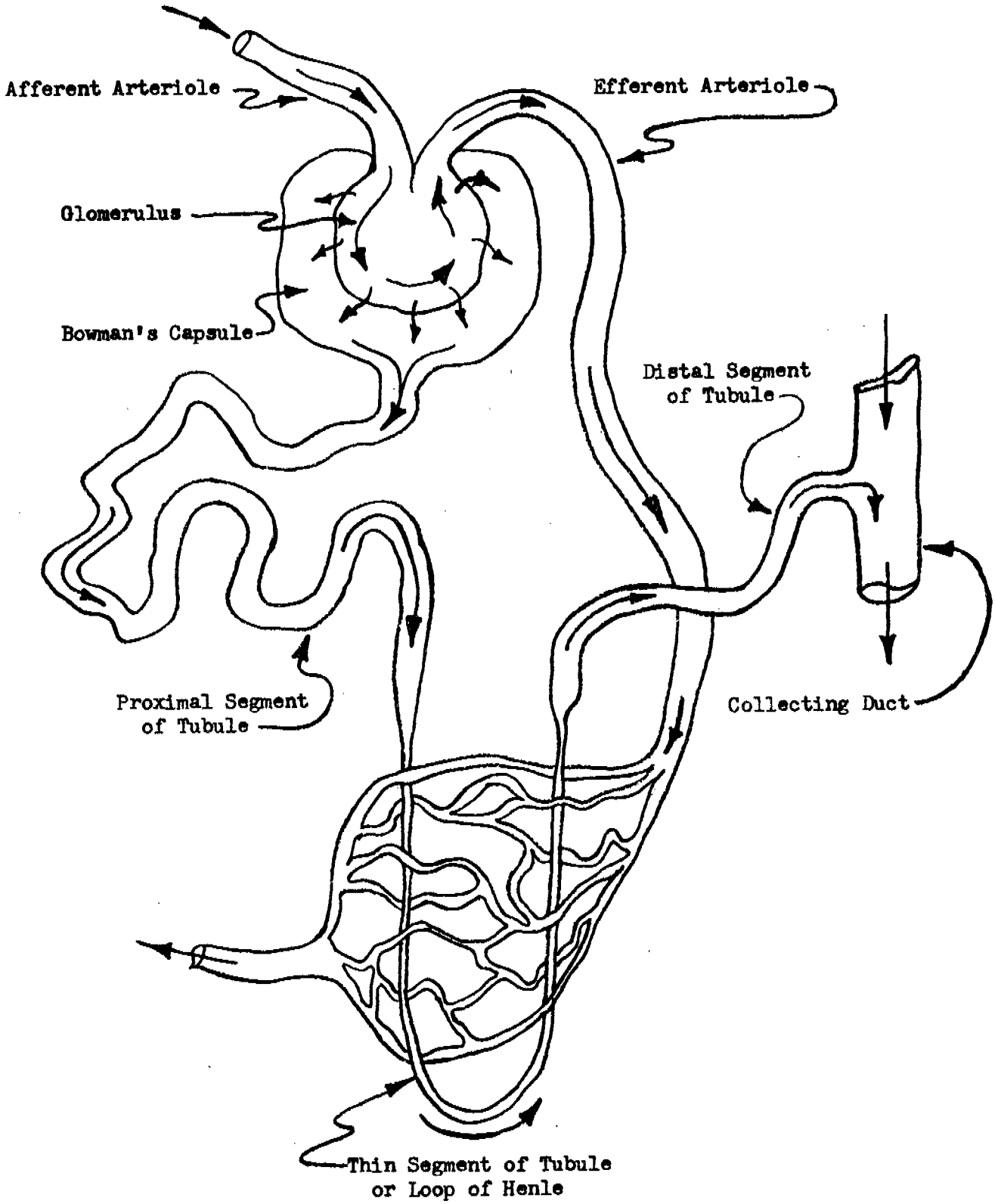
A. Kidney

The principal function of the kidney is to control body excretion so that electrolyte balance can be maintained. Smith (1) describes the human kidney as consisting of an integrated mass of individual units called nephrons of which there are approximately one million per human kidney. Each nephron performs the complete kidney process on a miniature scale.

The nephron (Fig. 1) has a spherical bundle of capillaries, called the glomerulus, which is surrounded by a spherical sac named "Bowman's capsule." The capsule is connected to a fine tubule. The tubule consists of three segments, called the proximal, thin and distal portions which follow the glomerulus in that order. Blood is supplied to the glomerulus, which under hydrostatic pressure, acts as an ultrafilter. Because of their large size, protein molecules, fat droplets and other complex substances are prevented from passing into the capsule and, thereby, are conserved within the body. The protein-free plasma enters the capsule and thence the tubule where it is modified by reabsorption or secretion of the constituents through the tubule wall in accordance with the demands of the body fluid. The reabsorption of sodium takes place in two stages (2). In the proximal tubule approximately 80 per cent of the sodium in the glomerular filtrate is reabsorbed into the body fluid, and the remaining sodium may be reabsorbed in the distal tubule. The mechanism of this reabsorption is of prime interest to the problem of saline water conversion. Many early workers believed this was accomplished by simple diffusion of ions due to electro-osmotic pressures. Calculations now show that the quantities transported in this manner are insufficient to have

FIG. 1

Diagram of Human Nephron



much significance in the kidney. Simple diffusion falls short of a quantitative explanation of kidney reabsorption by several orders of magnitude (3). Abundant experimental evidence also shows that there are substances, among them sodium, which move in a direction opposite than that which would be dictated by electro-osmotic forces. It is, therefore, necessary to conclude that energy must be expended in accomplishing this type of transport. Aerobic metabolism is generally accepted as the source of this energy. In the proximal tubule the reabsorbed sodium is accompanied by considerable quantities of water which traverse the tubule wall passively due to the osmotic pressure created by the reabsorption of the sodium. Chloride and other anions will also accompany the sodium passively because of electrostatic forces. The remaining fluid enters the thin segment of the tubule, called the loop of Henle, the wall of which is highly permeable to water. Here, osmotic equilibrium is established between the proximal urine and the plasma by the movement of water. The loops of Henle are separated from the proximal tubules but intermingled with the collecting tubules in such a manner that Cushny (4) said, "The arrangements certainly call to mind the regenerative heat exchangers used in the machines which liquefy gases; and one cannot help wondering whether some process of regenerative diffusion exchange may be at work." The forming urine passes from the thin segment into the distal portion of the tubule where the process of particular interest from the sodium chloride removal standpoint takes place. Here sodium is reabsorbed from the tubule into the plasma against very high concentration gradients caused by the continued reabsorption. If the demand of the body is high, distal reabsorption may remove the last trace of sodium from the urine. Moreover, this final separation of

sodium takes place at some point in the distal tubule where the wall is impermeable to water. Again, to satisfy electrostatic forces, chloride ions, together with other anions, accompany the sodium passively. Hence, an actual separation of sodium chloride and water occurs here.

A collecting system of ducts receives the urine produced by each nephron and, combining the minute flows, delivers the urine to the bladder.

The glomerular filtration rate in man averages 130 cc per min (5). The urine flow, however, averages 1.0 cc per minute in the normal man (6). Thus, approximately 129 cc per minute are reabsorbed in the tubules of the nephrons. During maximal water diuresis, the amount of urine produced may be as high as 20 cc per min. The volume of the human glomerulus averages 0.0042 cu mm. Thus, in each of the 2,000,000 glomeruli, approximately 0.001 cu mm of filtrate is formed per second. The blood flow through the two kidneys is approximately 1200 cc per min. In order to accommodate this flow, 0.01 cu mm of blood, two and a half times the volume of the glomerulus, must flow through the glomerulus each second. Values for the total filtration area of the two human kidneys have been calculated at 1.56 (7) and 0.76 sq m (8). Thus, the filtration rate for the normal healthy kidney is somewhere between 770 and 1580 cc per sq m per min. This is based on the total applied load to the filter. In terms of the amount of liquid penetrating the filter, the 130 cc per min cited above, 187 liters of water are filtered from the plasma each day. This is approximately 50 times the volume of plasma in the body and 15 times the volume of the extracellular fluid. In processing this quantity of fluid, the kidney tubules must reabsorb 185.5 liters of

water, 1100 gm of sodium chloride, 425 gm of sodium bicarbonate and 150 gm of glucose plus quantities of phosphate, amino acids, vitamin and other compounds (9). The final product of the kidney in this period of one day is 60 gm of urea and other wastes in 1.5 liters of urine. The energy expended and work performed by the kidney is solely in reabsorbing material from the forming urine as the energy for the mechanical filtration is supplied by the heart.

Evidence supporting the mechanical filtration function of the glomerulus has been obtained through renal "clearance" experiments. Clearance is defined as the ratio UV/B where U is the concentration of the given substance in the urine, V is the volume of urine formed per minute and B is the concentration of the substance in the blood. UV, then, is the amount of the substance excreted per minute. Then the clearance, UV/B, is the amount of blood which must be filtered to yield the amount of the given substance found in each minute's flow of urine. If effects due to reabsorption and secretion could be eliminated, the clearance would provide the true filtration rate. Substances, such as inulin and creatinine, which the tubules neither reabsorb nor secrete have been simultaneously administered to the blood stream and the clearances of the substances has been found to be equal. This fact is evidence that glomerular function is simple filtration since, if reabsorption or secretion were factors, it would be very unlikely that such dissimilar substances would be reabsorbed or secreted in the same quantities.

Glomerular pore size has been established by electron microscopy at approximately 50 Å in radius (10). Approximately five percent of the total surface of the glomerular membrane is occupied by these pores.

In addition to the absorption of electrolytes and other solutes by the kidney tubules, there is evidence that water is also actively reabsorbed (11). This is in addition to obligatory water reabsorption in which water passively accompanies the reabsorbed solutes in order to maintain osmotic equilibrium. The active process is promoted by the presence of antidiuretic hormone and may reabsorb as much as 20 cc of water per minute in man. This "faculative reabsorption" is believed to be confined to the distal portions of the nephron and probably the distal segment as well as possibly the collecting ducts. Faculative reabsorption is activated by the body water requirement. When this reabsorption is in abeyance, the urine flow is large and the urine is osmotically very dilute because of the reabsorption of sodium chloride in the distal tubule. Conversely, when faculative reabsorption is activated, the urine flow is small and the urine is concentrated. It has been shown (12) experimentally in the dog that water can be reabsorbed actively in the absence of antidiuretic hormone, but only under extreme stress and conditions that are not compatible with life (13). In terms of reclamation of saline waters, the process of electrolyte reabsorption and the process of water reabsorption are both of interest since either could be used to advantage.

Of primary interest to any practical application of renal principles is knowledge of whether renal action is controlled by the nervous system. If nervous control dictated kidney action, it would tremendously compound the problem of analyzing the already obscure mechanisms separating salt and water. Surgical experimentation with animals has been used to investigate the role of the nervous system in kidney

function. Hiatt (14) reporting on dog kidney experiments states that kidney circulation and function are probably not normally under tonic nervous control. Contrary to these findings, Kaplan, West and Foman (15), studying effects of unilateral division of splanchnic nerve on the renal excretion of electrolytes in dogs, cite evidence that denervated kidneys excreted sodium, chloride and water at increased rates. Trueta et al (16) found renal circulation to be subject to nervous control in that the flow may be shunted from its normal pathway in the cortex to a pathway through the medulla due to shock. This, however, does not indicate that such control is normally exercised. Smith (17) states that denervation has little or no effect upon kidney flow or urine composition.

The adrenal glands play an important part in sodium absorption and excretion in the kidney. The hormone aldosterone excreted by these glands, was shown by Luetscher and Curtis (18) to be related to the excretion of sodium. They found that aldosterone promotes the retention of sodium and the excretion of potassium. Luetscher (19) later quantified this relationship and showed an inverse proportion between the subject's sodium excretion and the quantity of aldosterone in the urine. Surgical experiments performed on dogs by Davis, Howell and Hyatt (20) induced cardiac failure by ligature construction of the pulmonary artery. Renal sodium excretion was studied in relation to hormone treatment administered to the failing animals. Desoxycorticosterone acetate was found to be capable of inducing complete sodium retention. Evidence that the adrenal glands can promote sodium balance other than by inducing sodium retention was cited by Ledingham (21). Studying nephrectomized white rats under various

conditions of fluid and salt administration, he found the adrenal to act in two additional ways in controlling the osmolarity of the expanded extracellular fluid volume. These were by withdrawal of sodium from skeletal muscle and by diminishing the intake of water. That the adrenal glands may also have a positive effect upon sodium excretion was indicated by Share and Hall (22). Dogs were adrenalectomized and continually injected with sodium chloride solution. It was deduced that adrenalectomy impaired sodium excretion, but this was believed due to reduced filter load. Urine volume was apparently determined primarily by the rate of solute excretion, with approximate direct relationship between urine volume and the sum of the potassium and sodium excretion rates. Despite the rise in sodium excretion due to the infusion of sodium chloride into the bloodstream, the rate of sodium reabsorption was rapid and high. A possible relationship between congestive heart failure and sodium retention was found by Mader, Yoshikazu and Iseri (23). Five patients showed high initial excretion of sodium followed by a sharp reduction. A sharp cellular uptake of sodium occurred with recovery.

Since the glomerulus will not pass protein and other large molecules, these molecules exert an osmotic effect on the "upstream" side of the membrane. Under conditions of osmotic equilibrium, this effect must be counterbalanced on the "downstream" side with the result that the electrolytes, although freely diffusible through the membrane, will not be distributed equally on both sides of the membrane. This effect is quantified by the Gibbs-Donnan equilibrium. Where the system contains a number of diffusible monovalent ions and an ionized salt of protein, NaR, the Gibbs-Donnan equilibrium may be written (24) in the form of an equality

of ratios of ion concentrations:

$$\frac{(Cl^-)_1}{(Cl^-)_2} = \frac{(HCO_3^-)_1}{(HCO_3^-)_2} = \frac{(Na^+)_2}{(Na^+)_1} = \frac{(K^+)_2}{(K^+)_1}$$

For mammalian plasma and glomerular filtrate, the Donnan factor for chloride, $(Cl^-)_f/(Cl^-)_p$, has been determined (25) as 1.02, and the factor for sodium, $(Na^+)_f/(Na^+)_p$ as 0.95 (26) at 6 gm of protein per 100 ml of plasma.

The average normal plasma is reported (27) to have the following concentrations and distribution of electrolytes.

Ion	Serum (mEq/L)	Serum Water (mEq/L)	Interstitial Fluid (mEq/L)	Intracellular Fluid (mEq/L)
Sodium	142	153	145	7
Potassium	4.3	4.5	4.4	155
Calcium	5.0	5.3	2.8	...
Magnesium	3.4	3.7	2.4	20
Chloride	104	112	118	3
Bicarbonate	27	29	30.5	10
Phosphate	2.3	...	...	...
Sulfate	0.6	...	...	...
Protein	16	...	...	...

The cells must therefore maintain a sodium imbalance of more than one to 20 with their environment, and, similarly, a chloride imbalance of nearly one to 40.

Kidney function adapts very rapidly to shifts imposed on the body sodium balance. Robinson and coworkers (28) exposed men to extreme heat and observed the effects of imposed salt deficiency on renal output and sweat. Salt retention of the kidneys began immediately, while a delay occurred before the sweat glands reduced the concentration of sodium chloride in the secretions.

The total electrolyte composition of the plasma is one of the most finely regulated features of the human body. The quantity of water retained in the body is dictated by the necessity for maintaining a constant osmotic pressure in the plasma. This, however, is directly related to, both subsequently and subordinately, the retention of sodium. Water ingested rapidly without sodium, is excreted completely in a few hours. However, the ingestion of normal saline solution does not result in increased excretion of water (29). The excretion of sodium is not independent, but is associated with the excretion of some anion, usually chloride, which follows passively to maintain electrostatic neutrality. Bicarbonate ions will interchange with chloride ions in accompanying the sodium. Chloride ions appear to play no specific role physiologically (30). Thus, they are not bound in any manner, but are free to move in satisfying electrostatic and osmotic requirements. Since sodium may be bound, equal quantities of ingested sodium and chloride ions may be excreted at very different rates because of readjustment of other anions and cations. As indicated above, it is mostly the chloride which interchanges rather than the sodium or other cations.

Other than that metabolic energy is expended in the process, little is known about reabsorption of sodium and chloride ions in mammals. It is believed, however, that independent mechanisms operate for each of the ions (31). In alkalosis, bicarbonate ions are excreted in preference to chloride ions, and, when the adrenal cortex is removed, sodium is excreted while potassium is not. The ability of the mechanisms to distinguish between sodium and potassium indicates that the processes must be highly refined.

The rapidity with which ion transport can occur in the kidney was demonstrated by Whittam and Davies (32). They incubated kidney cortex slices in a saline medium under aerobic conditions. During the first five minutes of incubation at 37° C, the slices increased their sodium content by 60 per cent. Maintenance of the sodium concentration gradient was found to be dependent upon the energy derived from respiration. Krakusin and Jennings (33) experimented with living rats by infusing veins with Na^{22} under various conditions of sodium balance and removing and sectioning the kidneys. Radioautographs were made of the sections and showed the progress of the isotope through the kidneys as a function of time. They found that sodium equilibrium between the intravascular and extravascular, extracellular fluid occurred within two minutes. A high percentage of sodium reabsorption was shown to take place in the proximal tubules. The uptake of sodium in sodium deficient rats was found to be more rapid and greater than in normal rats.

In all mammalian kidneys, blood reaching the tubules first passes through glomeruli. All vertebrates below mammals have a double blood supply, the afferent glomerular vessel and the renal portal system (34). Certain species such as the toadfish and the goose-fish do not have glomeruli. This means that all material excreted through the kidney must be absorbed through the tubule wall. This accounts for the fact that glomerular kidneys are able to excrete almost all foreign substances injected into the body while aglomerular kidneys can not. Such substances as ferrocyanide, cyanol, xylose, sucrose and inulin are not excreted, apparently because they cannot diffuse or be transported through the tubule wall.

As a consequence of accepting the evidence for the action of the glomerulus as an ultrafilter, it is necessary to conclude that reabsorption of salts, sugar, amino acids and other vital complex substances occurs in the tubules. Otherwise the body would be rapidly depleted of many of its vital constituents and death would quickly follow. With this strong foundation, research has been largely devoted to determining the sites and mechanisms of reabsorption rather than merely that it occurs. Richards (35) and his coworkers developed micromanipulative and chemical analysis methods to locate these sites. By being able to puncture single kidney cells of the bullfrog and extract the fluid, they determined concentrations of various substances in the glomerulus, the proximal and the distal tubule. Bullfrog kidneys were perfused with a solution of the substance to be studied and the change in concentration was followed through the course of the solution through the nephron. They found that glucose reabsorption was generally complete before the proximal tubule was traversed. It was shown that glucose was not reabsorbed in the distal tubule by perfusing the distal tubule with glucose and finding the same concentration being excreted. The method showed that the final and selective reabsorption of sodium occurs in the distal tubule. In the frog, the reabsorption of salts is greater than the reabsorption of water with the result that the urine of the frog is hypotonic as compared to that of mammals which, except under conditions of salt demand, is hypertonic. Phlorizin was found to inhibit completely the reabsorption of sugars while not affecting the reabsorption of water or chlorides. Other

metabolic poisons, such as cyanide and urethanes were found by David (36) to block all reabsorption. The microchemical technique suffers from the deficiency that it is impossible to determine by direct measurement how much water is filtered in any glomerulus and, consequently, how much water is reabsorbed in the tubule.

Hoshiko, Swanson and Visscher (37) perfused bullfrog kidneys with isotopes to establish control values of transport across the renal tubule. Aerobic energy system poisons were then administered and the effect on transport studied. The authors concluded that the reabsorption of sodium was reduced essentially to zero. The conclusion may be questioned, however, on the grounds that the controls showed a tendency toward reduction of reabsorption of sodium before the poisons were injected.

Evidence of the rapid transport of water in the tubule of the bullfrog kidney was cited by Swanson and his coworkers (38). Bullfrog kidneys were isolated surgically and perfused with D_2O. The D_2O moved freely across the renal tubule to establish equilibrium. Ninety percent of equilibration occurred before one percent of the proximal tubule was traversed.

Hoshiko (39) also perfused bullfrog kidneys to determine the effect of temperature upon reabsorption of sodium. He found the initial reabsorption rates to vary directly with temperature, and that these rates decreased immediately. The rate of decrease also varied directly with temperature. He stated that sodium reabsorption is a chemical reaction, or, more precisely, a complex chain of chemical reactions.

Other temperature experiments were performed by Smith (40) and Bass and Henschel (41). The former found little alteration in concentrations of serum electrolytes in dogs cooled to temperatures as low as 14° C. The latter found that cold increases the excretion of urine and the concentration of sodium chloride in it. On the other hand, they state that plants, insects and animals protect themselves from freezing by excreting water which increases internal salt concentration and lowers the freezing point. Most data indicates that heat causes an increase in plasma volume which probably reflects a shift of interstitial fluid.

Cheek and West (42) studied rats under a potassium restricted diet and performed ionic analyses on the sacrificed rats. They found an increase in body sodium content, indicating an attempt to substitute sodium for needed potassium. This may show some connection between potassium and sodium reabsorption at least insofar as the demand mechanism is concerned.

In experimenting with the goat, Anderson and McCann (43) found that electrical stimulation of the hypothalamus automatically activated the drinking mechanism of the animals. The stimulation also inhibited water diuresis. This may indicate the hypothalamus as being the seat of a control mechanism affecting the reabsorption of water in the kidney tubules.

B. Intestinal Mucosa and Secretory Glands

In order for sodium and other solutes to reach the blood stream, they must pass through the gastrointestinal wall. The amount of sodium lost from the body via the gut is small in the normal state (44). Except in cases of vomiting or diarrhea, almost all of the ingested sodium is absorbed into the blood stream. Cushny (45) detected a potential difference across the gastric mucosa in mammals and frogs. He associates this potential difference with active transport.

Absorption of sodium chloride into the colon and the distal part of the small intestine was observed by Budolfsen (46). He found that the amounts of sodium chloride absorbed increased in a linear fashion with the concentration applied. This relationship continued until the concentration of applied sodium chloride solution exceeded 1.25 percent. When the concentration exceeded 1.95 percent, the absorption decreased. The amount of sodium absorbed from solutions varying from 0.1 to 0.5 percent sodium content was approximately 65 percent of the administered load in the small intestine, and approximately 60 percent in the colon. When administered to the colon, approximately 60 percent of the sodium was absorbed by that tissue. Maximum uptake of sodium in the small intestine was 3.42 gm per hundred gm of tissue, and this was 2.64 gm per gm of tissue in the colon.

Weiss (47) studied potassium and sodium absorption in the duodenal cell with the electron microscope. He found evidence of physiological transport and suggested that cations may be somehow segregated by the cell in a manner that makes them osmotically inactive. This would

agree with the fact that the amount of potassium found in the cells would be ordinarily expected to cause considerable swelling if the potassium were osmotically active. The swelling, of course, would be caused by water entering the cell in an attempt to bring about osmotic equilibrium between the intracellular and extracellular fluids. However, observation shows that the swelling does not occur in the living cell.

It is generally believed that active transport of sodium occurs in the mucosa of the intestine and that aerobic metabolic energy is expended in the process or processes. Although still obscure, the mechanisms are probably similar to those operating in the kidney and other sites of active transport.

The ciliary epithelium of the eye is made up of two layers of what Davson (48) believes to be secretory cells. The cells of this tissue seem to excrete a continuous supply of aqueous humour which is essentially a solution containing the dissolved constituents in plasma. The main cation in the humour is sodium. The actual mechanism of secretion is speculative, but it is likely that the substance is elaborated within the cellular tissue and secreted by active transport.

The sweat glands also secrete sodium chloride. Active transport must occur here to explain the control that is exercised over sodium excretion through these portals. This was previously mentioned in discussing the work of Robinson and his coworkers (28) which showed that the sweat glands retained sodium chloride when men were subjected to extreme heat.

C. Amphibian Skin and Fish

A phenomenon which has been known and studied for many years is the ability of isolated frog skin to transport sodium chloride from its outer to its inner side. A section of skin placed as a membrane between a saline solution on the inside surface and a lesser or nonsaline solution on the outside surface will continue to transport salt "inwards" for several hours in vitro. This is an active transport process which works against the concentration gradient. As the result of the active transport of sodium ions inward, a measurable potential is generated across the skin. Zerahn (49) found that the potential across the skin depends on the oxygen supply to the skin. Since the potential is proportional to the rate of active transport of sodium which generates it, this is interpreted to demonstrate that the source of the energy expended in active transport is aerobic metabolism. In experimenting with a section of frog skin seven square centimeters in area, he obtained the following mean values.

Δ Na (uEq/hr)	Oxygen Consumption				
	uEq/hr		In % of Δ Na		ml/gr/hr Total
	Tot.	Net	Tot.	Net	
8.94	6.25	1.94	74	22.5	0.156

It is thus seen that the consumption of oxygen is approximately 20 percent of the amount of sodium transported when both are measured in equivalents. By placing distilled water in contact with the outside surface of the frog skin to eliminate sodium transport, Zerahn found that the amount of oxygen consumed by the skin was invariably less than

when sodium transport occurred. When oxygen was denied to the skin, the transport of sodium fell drastically in 20 to 40 minutes to about 10 to 20 percent of the aerobic value. His calculations showed that the net consumption of oxygen is large enough to supply the energy required for the transport. This energy was calculated as 1,340 - 2,680 gm-cal. Assuming one equivalent of oxygen to equal 25,000 gm-cal, and taking the net consumption of oxygen at the experimentally determined 20 percent of the sodium increment transported, this leaves a maximum of 5000 gm-cal available per equivalent of sodium transported. Since this amount of energy is sufficient to accomplish the transport, it appears that the calculated energy requirement of 1,340 - 2,680 gm-cal is not very far from the actual requirement. Work with skins from Rana temporaria, Rana esculenta and Bufo bufo also produced the 20 percent figure. The quantity was apparently independent of oxygen tension, and the administration of hormones.

On the other hand, Leaf and Renshaw (50) found that while ion transport decreased to 20-40 percent of its aerobic value in anaerobic tests, this reduced level of transport continued for some time. They assumed this to indicate that the transport process is not strictly aerobic, but that it is dependent upon metabolic energy that can be supplied by either aerobic or anaerobic pathways.

Huf and his coworkers (51) found that optimum uptake of sodium chloride by isolated frog skins occurred when the skins were maintained in a solution containing one to five microequivalents of potassium per milliliter. Under these conditions sodium chloride uptake averaged 0.90 microequivalents per square centimeter per hour. The potassium was transported from the inside to the outside of the skin, opposite to the direction of sodium chloride transport, at the rate of approximately 0.03 microequivalents per square centimeter per hour. It thus seems that the

presence of potassium may be essential in any solution from which frog skin transports sodium chloride.

Simultaneous measurements of active ion transport and oxygen consumption were made on isolated frog skin by Leaf and Renshaw (52). The number of sodium ions transported per molecule of oxygen consumed ranged from 2-13 with a mean of 6.82. Sodium transport was in the range of one to three microequivalents per square centimeter per hour. The accompanying quantities of oxygen consumed ranged from 0.2-0.4 micromoles per square centimeter per hour.

Ussing (53, 54) used tracers to study active ion transport through isolated frog skin. He found the sodium influx to be 0.1-0.3 micromoles per square centimeter per hour. The mean outflux was less than ten percent of the mean influx. The excess influx took place against a potential difference of 30-110 millivolts. This again confirms that the transport is an active one requiring the application of energy. Ussing attempted to locate the site of active sodium transport. Frog skin consists of two layers, the mesodermal chorion and the ectodermal epithelial layers. The chorion is built up of connective tissue containing blood vessels and smooth muscle cells. Just beneath the epithelial layer the chorion contains a number of chromatophor cells. The chorion is a meshwork and presents no serious obstacles to diffusion. Hence active transport would not be needed or utilized there. The epithelium consists of only two or three layers of cells. The active transport is ascribed to the single layer of cells nearest the chorion, the stratum germinativum whose cells are cylindrical in shape. If correct, this means that the cells of the

stratum germinativum extrude sodium and chloride ions through the basal cell membrane only. It is extremely interesting that Ussing has implicated a single layer of cells as the site of active transport in the frog skin. This is because workers in the past have ascribed the ability of the frog skin to transport sodium to the very complex structure of the skin. Ussing's finding tends to place active transport in the frog skin in or more nearly in the general category of other physiological mechanisms exhibiting active transport.

Linderholm (142) found that the current produced by the short-circuited frog skin is apparently due entirely to the flux of sodium ions. The current expressed in equivalents of gram ions (Faradays) per unit time is the same as the net flux of sodium ions. The net transport of sodium chloride for open-circuited skin was approximately 180 micromicromoles per square centimeter per second. This applied when the concentrations of the solutions on both sides of the skin were the same. The total energy released by oxidation was approximately 6.5 microcalories per square centimeter per second which is approximately 27 microwatts per square centimeter. Five to 10 percent of this was believed available for sodium transport. Tracer studies, however, indicated that only three to five percent of the energy released by oxidation is needed for sodium transport. The transport mechanism in the frog skin is probably similar to that in the kidney. Low pH inhibits active transport of sodium, and, therefore, so does the addition of carbon dioxide. The effects of drugs were explored and mersalyl was found to decrease transport while aminophylline increased sodium chloride transport from 174 up to 288 micromicromoles per square centimeter per second.

Frog muscle was found to behave similarly to frog skin by Keynes and Maisel (55) who state that extrusion of sodium from muscle could be accomplished by utilizing about 10 percent of the energy available from resting metabolism. Van der Kloot (56) confirmed this action of the frog muscle and found that the process could be prevented by previous exposure of the muscle to the irreversible anti-cholinesterase hexaethyl-tetraphosphate. It was concluded that cholinesterase is essential for active sodium transport and that a source of high-energy phosphate bonds is required to provide the energy although the means by which the energy is actually applied remained obscure.

Fish must also have a mechanism for absorbing salts from their environment. In the case of fresh water fish, the absorption is against the concentration gradient and active transport is indicated. It would be expected that, in the interest of conserving the accumulated ions, the skin of fish would be impermeable to the ions. Davson and Danielli (57) point out that this is not the case. The gold fish, for instance, can be readily depleted of its salts merely by continually washing it with running water. Here, again, is evidence that energy must be constantly expended by the organism to maintain its accumulation of ions when the internal concentration of the ions exceeds that of the environment. Krogh (58) showed that gold fish can absorb ions from the environment through the gills, including the absorption of chlorides from sodium, potassium, ammonium and calcium chlorides. This is in addition to an apparently independent mechanism for absorbing cations such as sodium. Thus, while fish absorb vital ions through their gills, it seems that active transport must also take place in the ion-permeable skin to prevent

the loss of these ions.

Salt water fish, as pointed out by Smith (59) have exactly the opposite problem. The environmental osmotic gradient is reversed from that of the fresh water fish and the higher osmotic pressure of sea water tends to draw water out of the body through the gills, oral membranes and the skin. The limited quantity of water formed by metabolism is not enough to replace the amount that would be lost in this manner. Hence, they must prevent the loss of this water or replace it from sea water from which they must also obtain water for urine formation. Salt water fish accomplish this by drinking sea water, absorbing the sodium chloride and water through the gut, and excreting the sodium chloride through the gills. This leaves relatively salt-free water in the body. The process requires that practically all the sodium chloride in the ingested sea water be excreted against an osmotic pressure gradient. Active transport is necessary to extrude the sodium, while the chlorine follows passively.

D. Plants

Like animals, plants must accumulate and maintain concentrations of vital ions. In order to do this, it seems that active transport, very similar to that exhibited by the animals, is required. Sutcliffe(60) states that the absorption and translocation of mineral salts in plants cannot be explained entirely in terms of known physico-chemical phenomena. The accumulation of ions against concentration and electrical gradients seems to depend on a mechanism deriving its energy from aerobic metabolism. Ion accumulation in the vacuoles of individual cells and in

the stele of roots and probably also the transport of minerals in the phloem are given as examples of active transport.

Hoagland and Davis (61) first showed that plant cells accumulate ions in their vacuolar sap at concentrations many times higher than that of the surrounding medium in which the plants grow. Electrical conductivity measurements indicated that the salts are present in a largely dissociated form so that the maintenance of the concentration gradients cannot be explained on the basis of binding of the ions to cell constituents.

Based on findings that the absorption of minerals in roots occurs most rapidly near the tips where growth is rapid, Steward and Preston (62, 63) concluded that ion accumulation is closely associated with protein synthesis. Growth may influence ion absorption indirectly by an effect on the internal concentration of ions. Jacques (64) observed that a species of algae increases its rate of ion absorption when the osmotic pressure of the sap was artificially reduced. The rate of water or ion translocation to other parts of the plant may also influence the rate of uptake in the root.

Salt uptake in plants seems to be more closely related to oxygen uptake than to carbon dioxide evolution. Lundegårdh and Burström (65, 66) first showed a quantitative relationship between the rate of oxygen absorption by isolated wheat roots and the rate of anion uptake. The release of carbon dioxide may continue when ion accumulation has stopped.

Hoagland and his coworkers (67) and Rosenfels (68) found that light influences the uptake of salt in Nitella and Elodea. Arisz (69) showed

that light stimulated the absorption of mineral salts by leaves of Vallisneria even in the absence of external carbon dioxide. Continuing his work, Arisz(70) demonstrated that when the leaves of Vallisneria were exposed to light they showed a fairly constant chloride absorption. The ions were secreted into the cell vacuole. Chloride could be accumulated in any part of the leaf exposed to light. Transport of the absorbed chlorides from the absorption sites to other parts of the leaf also required light. This transport was accomplished without loss of ions to the outer solution.

Overstreet and Dean (71) also found the absorption of ions from soil by plants to require the expenditure of energy by the plant. No accumulation occurred in the absence of respiration and other metabolic activities usually associated with growth. This was found true for both anions and cations. For each ion entering the plant, however, one ion must leave. Anions are absorbed in exchange for hydroxide and bicarbonate ions, and cations are exchanged for hydrogen ions.

Epstein (72) produced further evidence that the respiratory system is involved in ion transport by plants. The demand for oxygen for active transport was shown by growing plants in distilled water and measuring their respiration. When ions were added to the distilled water, the oxygen demanded by the respiratory system of the plant increased. Russell and Ayland (73) found, through isotopic competition studies, that diffusion was not significant in determining the rate of entry of ions into plant tissue. The transport process depended on exchange reactions at or near the cell surface.

It is thus seen that the pattern of ion transport in plants is quite similar to that in animals, at least at the present stage of

knowledge. Ion transport will be discussed further in the section entitled "Active Transport as a Cellular Phenomenon" since many plants of particular interest are unicellular.

IV. Active Transport as a Cellular Phenomenon

A. Unicellular Organisms

So far we have examined living organisms in descending order of complexity and found active transport energized by metabolic processes universally displayed in both the animal and plant kingdoms. It is surprising that at each level of life the organism can accomplish this whether or not it possesses a highly specialized organ, such as the human kidney, developed to perform the function. While the electrolyte balance problem of plants is uncomplicated by the process of bulk ingestion that confronts animal systems, except for glomerular action plants must perform the essentials of kidney function with regard to inorganic ions. For the purpose of practical application of physiological processes of ion transport to saline water conversion, the simpler the organism that can perform the function the more interesting it is. This is obviously because simpler organisms are easier to study or utilize even though the mechanisms of interest may be the same or as complex as those in higher forms of life. Accordingly, the active transport of plants was discussed in the preceding section. However, active transport of sodium, chloride and other ions occurs not only in the larger plants, but in the simplest complete form of life, the single-cell plant.

Conway and his coworkers (74, 75) found that yeast containing 60 millimoles of sodium per kilogram of the centrifuged cells were able actively and specifically to extrude sodium. The rate of extrusion was determined with isotopes and found to be more rapid than that in rat muscle. The half period for the extrusion in the yeast was approximately

35 minutes at 37° C and 100 minutes at room temperature. Excretion did not occur when 0.1 molar sodium chloride solution was added to the suspending medium unless a 0.1 molar solution of potassium ions was also added. It thus appeared that potassium ions exchanged inward for sodium ions extruded. The transport was found to be active and to require oxygen. It was believed that the transport mechanisms achieving the exchange of the two ions were different or, more specifically, that the transport was carried on by different phases of the same mechanism.

Active transport also occurs in unicellular photosynthetic plants in the algae group. Hoagland and Davis (61) demonstrated that the vacuolar sap of Nitella is predominantly a solution of salts corresponding in conductivity to a 0.1 normal sodium chloride solution. Fresh water Nitella were taken from a pond and the sap extracted for chemical analyses. The following data compares the concentrations of various ions, including sodium and chloride, in the sap to the concentrations of the ions in the pond water in terms of parts per million by weight.

Ion	K	Na	Ca	Mg	Cl	SO_4	PO_4	NO_3
Cell Sap	2120	230	410	430	3220	800	350	0
Pond Water	0.?	5	31	41	32	31	0.4	0.5
Conc. Factor...		46	13	10	100	26	870	...

Sap concentrations in hydrodictyon were reported by Blinks and Nielsen (76). The concentration factor of potassium in the sap over the concentration in the environment was approximately 4,000. Scott (77) studied the relationship of electrolyte environment to chemical composition of Chlorella Pyrenoidosa, a unicellular fresh water algae. He found that a deficiency of potassium, magnesium, sulfate or phosphate lead to cessation of reproduction. Mineral constituents of the cell could not be removed

by suspending cells in distilled water for two hours, indicating that the ions are either bound or that active transport, or both, are responsible. It is presumed this experiment was conducted in normal laboratory light.

Some marine unicellular algae vigorously extrude sodium. Living in sea water with a 0.498 molar sodium content, Valonia macrophysa and Valonia ventricosa were found by Osterhout (78) to have sap sodium concentrations that were 0.09 and 0.03 molar respectively. Other unicellular marine algae in the sea water concentrate sodium in the sap. Halicystis Osterhoutii, for instance, accumulated a sodium molarity of 0.557 in its sap when suspended in sea water of 0.498 sodium molarity. Other algae native to saline or brackish water concentrate sodium to higher degrees although a systematic presentation of this information was not found in the literature.

The algae are able to accumulate considerable quantities of salt and hold them against high concentration gradients or, depending on the species, they may resist the intrusion of various ions, again against high concentration gradients. They are organisms of considerable interest to the saline water conversion problem.

B. Cellular Activity

The similarity in the mechanisms by which ion transport is actively accomplished extends not only from organism to organism, but also to the cells of each organism. By means of its kidneys, intestines, secretory glands, gills, skin, roots or other organs or parts, the organism must regulate its total electrolyte composition by accumulation from and excretion to the environment. The organism must go one step further, however, and distribute these electrolytes between its extracellular and intracellular fluids. Each individual cell must maintain

vital differences in electrolyte concentrations between its fluid and the environment of extracellular fluid constantly bathing the cell. Thus, despite the fact that highly specialized organs may transport ions for the total body balance, physiological ion transport is a universal cellular process in macro organisms just as it is in unicellular organisms. The mechanism by which cells transport an ion actively is frequently called a "pump." Thus the human erythrocyte is said to have a "sodium pump" among other types of pumps. Wilde (79) found the sodium influx maintained by this sodium pump to be approximately 3.08 milliequivalents per liter of cells per hour. The pump was operating at 60 percent capacity when the external sodium concentration was 143.5 milliequivalents per liter of medium. The computed energy expended by the pump was 6.0 calories per hour per liter of cells. He concluded that the energy requirement of the sodium pump consumes much less of the total available energy than was first thought.

An interesting fact pertinent to the object of this study was reported by Greig and Gibbons (80). When human blood was refrigerated, the erythrocytes lost potassium and gained sodium. Upon warming the erythrocytes to 37° C in an appropriate substrate, the reverse occurred. The fact that the reaction was shown to be reversible indicates that the pump may be stopped by deprivation of energy, but that, once again supplied with energy, it will again function. This may mean that the pump action can be controlled so that cells might be used to transport sodium from one solution to another.

Hunter and his coworkers (81) allowed chicken erythrocytes to incorporate sodium from solutions 0.9 N in sodium chloride. The

incorporation rate increased with time. At the end of 40 hours, the cells had removed as much as 160 milliequivalents of sodium per liter of cells. Assuming that chloride followed passively, this would be 9.35 gm of sodium chloride removed per liter of cells over the 40-hour period.

V. Biological Membranes

A. The Cell Membrane.

The site of active transport in cells is the cell membrane. Conway (82) and most investigators believe that the transport is accomplished by enzymes operating at the membrane surface. Evidence that some enzymes are present on the exterior of the cell membrane is cited by Rothstein (83). The enzymes apparently forage the environment for specific ions and somehow catalyze the transport of the ions through the cell membrane.

There is considerable interest in the special physical properties of the membrane that are associated with active transport. Electrical conductivity is a tool that has been used in investigating the membrane. Hober (84) introduced the use of conductivity measurements when he found that, although the interior of the cell had a high conductivity, the conductivity of the cell membrane was very low. From membrane resistance measurements it has been calculated (85,86) that the cell membrane is approximately 30 - 50 Å thick. This was based on an assumed dielectric constant of 3, which was believed to correspond to the type of material composing the membrane. Some investigators believe the dielectric constant might be considerably higher and have, therefore, estimated the membrane thickness as high as 150 Å. For the sake of comparison with synthetic membranes, it might be mentioned that the thinest collodian membranes made are approximately 1,000 Å thick.

The high resistance of the cell wall is due to the extremely small permeability of the membrane to ions. Under an applied potential, ions move through the cell membrane in a period of time that is about equal

to the time they require to move through a 100 cm layer of 0.1 normal potassium chloride (87). This indicates that the membrane is about 10^9 times less permeable to ions than the potassium chloride solution.

The death of a cell is accompanied by a sudden and great increase in permeability to ions. The trout egg, for instance, is impermeable to salts and normally exhibits a clear yellow color. After death, however, the internal salts leak out rapidly leaving the cell with a cloudy white appearance due to the precipitation of yolk globulins. This reaffirms the conclusion that metabolism is associated with the ion permeability and transport properties of the cell membrane. Further support may be deduced from the ability of some narcotics to inhibit ion transport. The means by which narcotics produce this effect, however, is still a matter of considerable debate among investigators.

With many variations, three principal types of physical models of the cell membrane have been offered by investigators. The first, and one that seems to find increasing support (88), visualizes the membrane as being porous. The wall consists of protein and lipoid molecules arranged in long, thin "flakes" which are then stacked to form the wall. The structure is discontinuous, having small slits with special properties. Transport takes place through the slits. The second model is that of a continuous, homogenous membrane (89). The transport is accomplished by moving the transported material through the membrane much in the manner of a solute moving through a solution. The third model proposes a nonhomogenous or "mosaic" structure for the membrane (89). This hypothesizes that the entire membrane is not permeable, but that only isolated patches are. The model requires a very complicated structure, but is believed by some workers

to explain the low permeability of the membrane to diffusion on the basis that the permeable patches constitute only a small portion of the total cell membrane area.

B. Mitochondria.

As analytical techniques have improved, smaller and smaller components of cells have been investigated. In recent years evidence has been accumulated to the effect that mitochondria, structural portions of the cell responsible for almost all of its respiratory metabolism, are themselves capable of considerable ion transport (90). The mitochondria have been variously described as consisting of or containing more or less mobile granules within the cell capable of contact at the cell wall, or as highly specialized structures at the cell wall. It is believed by DuBuy (91), Green (92), Hogeboom (93) and their coworkers that enzyme systems are located on the mitochondria. Mitochondria can be fractionated from the rest of the cell and their properties studied.

Bartley, Davies and Krebs (94) believe that mitochondria are the basic units of secretory and absorptive activities of the cell. Bartley and Davies (95) isolated mitochondria from sheep kidney cortex homogenate and found them to maintain concentration differences between themselves and the external fluid. While varying with experimental conditions, the concentration of sodium in the mitochondria was found to be approximately 26 times that in the suspending medium. Since the mitochondria are cell components, this means that there are concentration gradients within the cell.

Evidence that complex anions are bound by mitochondria was presented by Jacobs (96). This binding produced significant changes in

the oxidation-reduction potentials of the mitochondria. Weiss (47) found that cells transporting large amounts of cation contain large numbers of mitochondrial granules. The proximal renal tubule of the kidney was cited as being particularly rich in such granules. The mitochondria of cells not engaged in extensive ion transport did not contain as many granules. When an excess of sodium or potassium was added to the transport load, the number of mitochondrial granules significantly increased.

Huff, Wills and Arrighi (51) believe that the source of oxidative energy is localized in the mitochondria. They also found that potassium absorption appeared to be related to sodium transport in the mitochondria.

Mitochondria in plant cells were studied by Robertson and his coworkers (97). They found mitochondria in carrot and beet tissue to accumulate both cations and anions. More free sodium and potassium ions were concentrated in the mitochondria than in the suspending solution.

Much work needs to be done in clarifying the role and structure of mitochondria. It may be that the mitochondria are the ultimate functional units responsible for active transport.

VI. Theories and Mechanisms of Ion Transport

A. Passive Processes

It has been observed that a fraction of the total volume of various cells is accessible to electrolytes by free diffusion. Briggs and Robertson (98) found this uptake to be reversible and to take place relatively rapidly. The volume into which such diffusion can occur is thought to include the wet cell walls, intracellular spaces and, according to work by Robertson (99), at least some parts of the cytoplasm. Hope and Stevens (100), Butter (101) and Cowie and Roberts (102) produced data to indicate that it may amount to as much as 25 percent of the total volume of certain plant roots and to over 70 percent of the total volume of the microorganism E. coli.

Another passive process of ion transport is that of ion exchange. Part of the volume into which free diffusion takes place is occupied by immobile, dissociable groups fixed to the cell walls and to indiffusible molecules in the interior of the cell. These give rise to ion exchange, mainly cation exchange under normal physiological conditions, with the electrolyte in the external medium. Electrolyte uptake by this mechanism has been emphasized particularly in studies with plant roots by Epstein and Leggett (103) and Epstein and Hagen (104).

The passive processes have been mentioned because they do account for a portion of ion transport in living cells. As has been mentioned previously, however, they do not account for more than a small fraction of the transport rates demonstrated by cells, nor can they account for the maintenance of the high concentration gradients associated with cells.

B. Active Transport

Scientific inquiry into the precise workings of physiological mechanisms of ion transport has not yet removed the processes from the realm of speculation. Of the many theories proposed, none has been able to assimilate the observed data on ion transport into a single rationale. Some of the more prominent theories of "active transport" will be discussed. Active transport has been very specifically defined by Rosenberg (105) and other workers, but is generally accepted as referring to electrolyte uptake requiring the expenditure of energy derived from intracellular metabolism.

The "carrier" theory proposes, with considerable supporting evidence, that the active transport of ions from the exterior to the interior of the cell is accomplished by combination of the ion transported with a carrier substance in the membrane. The membrane is not permeable to the free ion. The carrier accepts the ion into the membrane by forming a complex with it. Expending energy, the carrier transports the ion through the membrane and then dissociates from it. The ion is thus placed on the interior side of the membrane and may not diffuse back across it despite the increasing electrochemical potential in the outward direction. The carrier returns to the "upstream" face of the membrane to repeat the cycle. Three of the more cogent facts suggesting such a mode of transport are:

1. Ions taken up by active transport are not readily exchangeable with other ions of the same species in the external medium, i. e., back diffusion and isotopic exchange are greatly hindered, indicating the presence of a barrier poorly permeable to free ions.

2. The rate of accumulation of a particular ion increases as the concentration outside is increased, but reaches a limit indicating

saturation of the carrier with the transported species of ion.

3. The specificity of ion uptake during active transport also suggests combination with a carrier molecule as an initial step.

An experiment devised by Osterhout (106) demonstrated the principle of the carrier theory. A layer of guaiacol (HG), representing the protoplasmic surface layer of the cell, separated two aqueous solutions, representing the internal fluid of the cell and its external environment. Potassium hydroxide was introduced into the "outside" solution. The potassium ion, per se, could not diffuse into the "inside" solution because guaiacol is essentially impermeable to it. However, the guaiacol reacted with the potassium to form potassium guaiacolate ($HG + K^+ + OH^- \longrightarrow KG + H_2O$) which diffused to the inside interface of the guaiacol layer. Carbon dioxide was continuously bubbled into the inside solution simulating its production in the living cell and providing bicarbonate ions with which the potassium ions might associate in solution. The potassium guaiacolate reacted with the carbonic acid in the inside solution to release potassium in association with the bicarbonate and to reform guaiacol ($KG + H_3O^+ \longrightarrow K^+ + HG + H_2O$). Because of the low pH maintained in the inside solution, the potassium ions had little opportunity to compete with hydrogen ions for the guaiacol and thus could not escape back across the "membrane" in appreciable quantities. The result was that the potassium concentration in the inside solution increased until it considerably exceeded the potassium concentration in the outside solution. Since no expenditure of metabolic energy impelled the potassium guaiacolate from the outside to the inside interface of the guaiacol layer, the rate of potassium transport would be limited to the rate of diffusion of potassium guaiacolate through the layer. A further interesting

aspect of this model was that potassium was taken up in preference to sodium when both were present outside.

The energetics of systems of the general type designed by Osterhout have been investigated theoretically by Rosenberg (107) by means of a thermodynamic analysis. It is supposed that components A and B are present in two phases, i and o, separated by a phase M. It is first assumed that M is impermeable to the solvent and to A and B, but permeable to compound AB. It can be shown by thermodynamics that an equilibrium can exist when the difference in the chemical potential of A between i and o is equal to the difference in the chemical potential of B between o and i. But if phase o is connected to a B-donator and phase i to a B-acceptor, the complex AB will be transported from o to i, thus producing an accumulation of A in i opposite to its gradient of chemical potential. In this simple system, B has the function of a carrier, but the cooperation of the surroundings is necessary, in the form of a source and a sink for B, in order for accumulation to occur. To couple accumulation to a chemical reaction, as is believed to occur in biological systems, a nondiffusible substance C may be added to phase i which is able to react with B to give the indiffusible compound BC. If A is the component to be accumulated, the energy for the transport would be derived from the reaction $B_o + C_i \longrightarrow BC_i$.

Another case of transport in a stationary system, the "osmotic diffusion pump," has been proposed by Franck and Mayer (108). In this model the connecting link between the inside and outside solutions is a solution compartment bounded at either side by a membrane. The connecting link is assumed to contain two solutes, A and B, B being an n-fold polymer of A. It is further assumed that the diffusion coefficient of A is less

than n-fold greater than the diffusion coefficient of B. Thus the same quantity of material can diffuse more readily in the B form than in the A form. The conversion of nA to B and vice versa does not take place within the connecting link but nA ⟶ B is catalyzed to equilibrium at the membrane bounding the outside solution. The supply of some other material at the membrane bounding the inside solution dissociates B to nA and forces the reaction to produce more nA than would be produced under normal equilibrium conditions due to some change in the supplied material which releases free energy. Franck and Mayer conclude that in the system A will be continuously cycled by diffusion from the inside membrane wall where it is produced to the outside membrane wall where it is converted catalytically to form B. The latter compound then diffuses back to the inside wall to be reconverted to dissociated form A. Because of the assumptions about the relative diffusion coefficients of A and B the concentration gradient of B will be less than that of A, so the total concentration of solute particles will be less at the outside membrane wall than at the inside. If then the membranes are permeable to A but not to B, a dynamic equilibrium is set up in which the chemical potential of the solute A will be higher in the inside solution than in the outside solution. A supply of free energy to the connecting link is required to maintain a dynamic equilibrium, but solute may be transferred from outside to inside if the rate of the reaction supplying the free energy is sufficiently increased.

The authors point out that the formation of polymers is not essential to the theory. For example, sodium chloride may be concentrated

by moving with the hypothesized soluble molecule, $H_nR(OH)_n$ from the outside to the inside membrane walls. When catalyzed at the membrane walls the following reaction takes place: $nNa^+ + nCl^- + H_nR(OH)_n \rightarrow Na_nRCl_n + H_2O$. The molecule Na_nRCl_n must then diffuse to the inside membrane where the reaction is reversed by the expenditure of free energy of some other reaction. If the membranes are assumed to be permeable to Na^+ and Cl^- then these ions would accumulate inside. The system proposed would work more effectively if a number of cells operated in series. Detailed methods of computing the necessary energy output for various model systems are given. Under favorable conditions the ratio of expended free energy to that gained as useful work may be as low as three.

Numerous authors have attempted to formulate theories which apply the carrier hypothesis realistically to biological transport systems. Most of these have had a short life as new experimental facts were obtained and none have achieved any universal acceptance. Those chosen for presentation here seem to have some consistency with experimental findings or have novel features which appear worthy of consideration. It should be mentioned, however, that not all investigators believe that a cell membrane with special transport properties for ions is necessary. Ling (109) has given reasons for thinking that the interior of at least muscle cells comprises an assembly of fixed charges on which ions, mainly potassium, are held by coulombic forces. Metabolic energy is required indirectly to maintain the cellular proteins in an extended state in which their ionic groups are available. Shaw and Simon (110) accept this view for both nerve and muscle.

Lundegårdh (111) has proposed a theory of salt accumulation of plants based on "anion respiration," which has some features in common with the more theoretical models described above (112). This theory was suggested by the observation of Lundegårdh and Burström (65) that a quantitative relationship existed between the rate of oxygen uptake by isolated roots and anion absorption. No such relationship was found for cation absorption. Lundegårdh, therefore, assumes that the absorption of anions and cations are independent of each other to the extent that different mechanisms must be responsible for their uptake. The anion uptake is considered to depend on a portion of the respiration of the cell called "anion respiration." The anion respiration is believed to be different from the remainder or "ground respiration" of the respiration of the cell. This assumption is based in part on the observation that inhibitors of enzymes stop salt uptake but leave the ground respiration almost unaffected. This indicates that anion respiration is catalyzed by hemin compounds, probably cytochromes, and that the ground respiration is carried on by a different enzyme system. Thus, in the Lundegårdh theory, the cytochromes assume the role of carriers.

When the iron atoms of the cytochromes are oxidized, their valence increases from plus two to plus three, at the same time giving up one electron. Thus the trivalent iron atom attracts one more anion than the bivalent iron atom. If the cytochrome enzyme system is situated as a bridge across a cell boundary, electrons may move out of the cell in exchange for anions, provided a redox gradient exists between the inside and outside of the cell. The transference of an electron between two iron atoms moves one anion from the oxidized to the reduced forms

of the enzyme. The process may be regarded as a streaming of anions between a positive pole represented by the cytochrome and a negative pole represented by the cytochrome oxidase. If some of the protons produced at the positive pole are consumed in the excretion of a free acid of the anion, metallic cations will substitute for them in the stream of positive electricity required to complete the circuit. In this way, cation excretion may be accomplished. At present, however, little is known or theorized about the possible participation of the cytochrome system in the active transport of cations. Other possible means of cation absorption include transport along the electrochemical gradient set up by the actively transported anions, and simple exchange for hydrogen ions in the cell.

The anion respiration theory has received considerable experimental support by plant physiologists such as Robertson (113), Wanner (114) and Lundegårdh (111), but it must be admitted that no evidence exists for the cytochrome organization assumed. In addition, there is no experimental evidence indicating that the theory can account for the selective uptake of ions of the same sign of charge.

A "redox-pump" model of ion transport has been advanced by Conway (115, 116). Hydrogen ions from metabolism may be accepted into a system of reduced cytochrome $\rightleftarrows$ oxidized cytochrome. The hydrogen is then transferred within the membrane to a metal respiratory catalyst with a higher redox potential, and hydrogen ions are released. Hydrogen is brought across the cell membrane either by diffusion through the membrane or by rotation of the catalyst in a very thin membrane to present

the hydrogen to the outer interface. The electrons are retained from the hydrogen so that hydrogen ions are secreted by the cell. The retained electrons are returned to the cell by a second metal catalyst and transferred to oxygen. Anions could be removed from the cell by the potential gradient created by the active transport of hydrogen, or they might be transported actively. In the latter case, the transport is linked with hydrogen transport through absorption of the anions on the inside interface of the membrane by an oxidized metal catalyst followed by movement to the external side of the membrane. In the case of fermenting yeast, sodium is actively removed from the cell. Conway theorizes that a special sodium carrier in the cell accomplishes the removal of this cation.

Leaf and Renshaw (50, 52, 117), on the basis of extensive laboratory work, take exception to the redox-pump theory. They found that the theoretical rates of ion transport and oxygen consumption calculated from the model were insufficient to account for the actual rates observed in an experiment designed to test the theory. In work with frog skin, they determined that the number of sodium ions transported per molecule of oxygen consumed averaged 6.82. This is significantly higher than the 4.0 ratio which would prevail under the redox-pump hypothesis. In addition, as previously stated, they found that some ion transport continued under anerobic conditions and concluded that this conflicted with the redox-pump theory.

The possibility that temperature gradients might cause active transport of solute and solvent across membranes has been discussed by Spanner (118). This author believes that temperature may be of some importance in producing active water transport provided the membranes

in question have sufficiently high heats of transfer. Little information concerning the heat of transfer across either natural or artificial membranes is available. The author thinks that more promising explanations for solute transport are being developed. Of course, the effect of temperature in increasing metabolism would influence the rate of active transport if the energy were metabolically derived. Scott and Hayward (119) demonstrated this effect with the algae Ulva lactuca by noting an increase in the transport rate of potassium when the temperature was raised from 20° C to 30° C.

A "protein flexure" theory by Goldacre (120) proposes that ions and water might be transported by the folding and unfolding of protein chains in the cell membrane. The property of protein to absorb solutes and water appears to vary depending on the physical configuration of the protein. Proteins have been shown to absorb many times more dye in the unfolded monolayer state than they do in the folded globular form. Thus, unfolded proteins might absorb ions or solutes from the environment and release them inside the cell by subsequent folding which withdraws the available sites from the ions. Goldacre cites his work with amoeba and with plant roots which suggests that, during activity, proteins at the cell or vacuole wall are constantly streaming along the interface, becoming spread and unfolded at one end and folded at the other. In this process, dyes are seen to be taken up by the unfolded protein molecules and released inside the cell (or vacuole) when they fold. The author speculates that water might also be transported by such a mechanism due to different degrees of hydration of proteins in the folded and unfolded states.

VII. Active Transport of Water

An alternative to the active transport of ions in achieving saline water conversion would be the active transport of water. Obviously, any method that could remove the water from the salt solution would be of at least equal interest with processes that remove salt from water. It has been pointed out that, under certain conditions, water may passively accompany actively transported ions in order to maintain osmotic equilibrium. This action would work to the detriment of saline water conversion possibilities since it would offset or tend to negate the effect of the active transport of the ions. If, however, water could be actively transported independent of any ion transport, a potential conversion method would be indicated. There is some evidence, part of which has already been discussed in the section on kidney function, that the active transport of water occurs in physiological processes, although this is a matter of considerable discussion.

Franck and Mayer (108), in their report of the osmotic diffusion pump, state that the system embraces the active transport of water. The osmotic diffusion model of active water transport has been reviewed by Bayliss (45). He calculated that the theory would require the supposition that the diffusion constant of the osmotically active substance within the cell be 1,000 times smaller than that of most ordinary substances in free solution. He concludes, however, that this is not an altogether unreasonable assumption.

It is believed by many investigators, including Smith (121), that active transport of water must occur in the distal tubule of the kidney in order to produce a hypertonic urine. This is in

addition to the obligatory reabsorption of water which occurs in the proximal tubule. The active reabsorption is the means by which the body may regulate its water requirements just as the active reabsorption of ions allows for the regulation of those substances. Smith (122) states that the active transport of water is promoted by the presence of anti-diuretic hormone.

Danielli (123) examined the problem of explaining the excretion of hypertonic urine by the kidney and concluded that no mechanical system based on anomalous diffusion can satisfy the facts.

One of the foremost proponents of a "water pump" is Robinson (124, 125) who believes that water is extruded actively as fast as it diffuses into a cell under the influence of the osmotic gradient. He argues for the occurrence of active water transport in the human kidney and in all organisms down to single-cell plants and animals. While admitting that the mechanism is obscure, Robinson, nonetheless, contends that the evidence for active water transport makes a good case. He cites the work of Kitching (126,127,128) to the effect that the vacuoles of fresh water protozoa seem to excrete water which diffuses in due to the higher osmotic pressure of the environment. Metabolic poisons which stopped the movements of the vacuoles caused the organisms to swell indicating that water was entering the cell unopposed by the water pump. By such techniques, the external osmotic pressure required to prevent swelling when the vacuoles were not functioning was determined. These determinations on protozoa indicated that the osmotic pressure acting on the body wall due to the excess of concentration of ions inside the cell over its environment were in the range of 170 - 850 mm of mercury.

Unopposed, this pressure would destroy the membrane. Robinson believes the most satisfactory of possible explanations for this condition is that water rapidly enters the cell to satisfy the osmotic pressure, but that it is just as rapidly pumped out to prevent the cell from swelling and rupturing. Water uptake in kidney slices is also cited as evidence by Robinson. Those arguing against the water pump contend that water uptake in kidney slices is due to water passively accompanying actively absorbed sodium. However, when respiration was inhibited in kidney slices in sodium-substituted media, the water content of the slices continued to increase. This indicates that an independent process is required to explain the continued uptake of water.

Another mechanism by which active transport of water may be accomplished is the protein flexure theory of Goldacre (12) discussed in the previous section. This model may function to transport water as the result of different degrees of hydration being associated with the folded and unfolded states of the protein. Unfolded protein saturated with water of hydration may move to another location and contract, shedding water as hydration sites are withdrawn into the fold.

VIII. Phosphorylation As Energy for Active Transport

Most investigators believe that the source of energy for active transport is derived from aerobic metabolism, but very little is known about the actual mechanism by which the required energy is made available to the cell. While this is probably the most obscure facet of active transport, it is also one of the most important.

Investigations of the biochemistry of cellular metabolism have led to the recognition of certain compounds having phosphate linkages of the pyrophosphate type, termed "high-energy phosphate bonds," which liberate unusually large amounts of energy on hydrolysis. These bonds are produced as a result of the oxidation of foodstuffs. They play a central role in coupling the energy derived from oxidative metabolism to the many physiological processes requiring energy such as muscular contraction and the synthesis of cellular components. One of the most versatile of these high-energy phosphate bonds is that found in adenosine triphosphate (ATP).

In view of the generally essential function of high-energy phosphate bonds, it was natural that a connection between them and the production of osmotic work by the cell should be sought. Although much work has been done with a wide variety of biological systems and many interesting hypotheses advanced, the mechanism of the transformation of the chemical energy to osmotic work has not been revealed. Only a few of the many contributions made toward the solution of this problem will be mentioned here.

It has been known for some time that phosphorylation of certain sugars seemed to be necessary for their absorption by animal cells.

Hoagland (129) was one of the first to speculate on the possible utilization of high-energy phosphate bonds for the accumulation of electrolytes. Mudge (130, 131), similarly, found evidence for the involvement of phosphorylations in the accumulation of potassium in kidney slices.

Steward and Street (132) suggested a hypothetical mechanism for moving electrolytes into plant cells in which compounds containing energy-rich bonds produced by oxidative metabolism functioned as ion carriers. In regard to this theory, Lardy (133) brought evidence against any one-to-one relationship between the number of ions accumulated and the energy-rich bonds utilized, at least insofar as brain tissue is concerned. His calculations showed that a highly inefficient operation would result if one energy-rich bond were required for each potassium taken up against a concentration gradient, since this would require about one-third of the total number of high-energy phosphate bonds produced. This is considered an excessive drain on the energy available to the cell in view of the many other essential, energy-depleting functions which must be performed.

The application of a recent development in physics, however, has added somewhat to the evidence supporting the phosphorylated carrier theory. Using nuclear-spin resonance techniques developed by Wertz (134) and Andrew (135), Jardetzky and Wertz (136) investigated sodium transport. They determined that some metabolites such as lactate, pyruvate and citrate did form specific complexes with the sodium ion. While these metabolites do not contain phosphorous, they are intermediates in the chain creating phosphorylated compounds. Hence, sodium complexes might also be formed with the latter. No such complexing effect was found between sodium and

formate, acetate, benzoate or chloride ions.

Much of the experimental evidence pointing to a connection between metabolic reactions and electrolyte accumulation has been gathered by the use of specific inhibitors of certain of these reactions. For example, dinitrophenol has been useful since it was found to prevent the production of energy-rich phosphate bonds as cited by Loomis and Lipmann (137).

Fuhrman (138) has made a study of the effects of inhibitors on the active transport of sodium in the frog skin. He also found that dinitrophenol reduced active sodium transport and he expressed the belief that high-energy phosphate compounds are involved in this accumulation process.

Robertson, Wilkins and Weeks (139) reported that dinitrophenol reduced or eliminated the uptake of electrolyte by carrot tissue without interference with respiration. Machlis (140) and Ordin and Jacobson (141) have made extensive studies of the effect of various metabolic inhibitors on ion absorption and respiration by plant roots. They found that inhibitors of glycolytic reactions and of the Krebs cycle also inhibit uptake of electrolyte from the medium. This indicates the connection between phosphorylation and uptake since the Krebs, or tricarboxylic acid cycle, is a succession of oxidative steps involving the removal of hydrogen from substrates with the simultaneous formation of high-energy phosphate bonds. Ordin and Jacobson believe some steps of the Krebs cycle, or closely associated reactions, are necessary to furnish the energy for both cation and anion uptake. They also found that dinitrophonol and arsenite, an inhibitor which acts as a phosphate replacement, inhibited

ion uptake, again suggesting that phosphorylation reactions may play a role. They speculate that ATP might be involved in ion accumulation by reacting with intermediates of the Krebs cycle to form or destroy ion carriers. In this scheme metabolism would be required to provide ATP and the intermediates to interact with ATP.

IX. Comparison of Physiological to Artificial Processes of Saline Water Conversion.

A. Surface Area

One of the prime criteria that determines whether or not physiological mechanisms of ion transport should be seriously considered in saline water conversion is the relative size of the production facilities per unit product. Even though physiological processes may function with extreme energy efficiency, if an enormous installation would be necessary to produce a nominal amount of fresh water, initial costs and maintenance would prohibit any industrial application. While the information that has been cited in this report indicates that it is quite likely that good energy economy is the case, the small total quantities of salt transported physiologically in any organism raises the question of practical size. Since it has been shown that transport occurs at the cell surface, the important consideration is the rate of sodium chloride transport per unit area of active surface.

Data on such rates in frog skin have been determined. Adjusted to grams x 10^6 per hour per square centimeter of skin in order to permit comparison, values are: 52.6 (51), 38.0-60.6 (142), 5.9 - 17.6 (54), 74.8 (49) and 58.5 - 175.5 (52). Using 50 x 10^{-6} gm/hr/cm^2 as a value well within the range, the surface area required for complete desalting in one hour of one gallon of sea water containing 30,000 ppm sodium chloride may be calculated.

$$30{,}000 \text{ ppm NaCl} = \frac{3 \times 10^4 \times 8.33 \times 454}{10^6} = 113.4 \text{ gm NaCl/gal } H_2O$$

$$\frac{113.4}{50 \times 10^{-6}} = 22.7 \times 10^5 \text{ cm}^2 = 227 \text{ m}^2 \text{ required.}$$

This is a prohibitively large surface area for any practical application of the principle to a continuous surface in one plane. As compared to the frog skin area requirement, the synthetic membrane used in electrodialysis could accomplish the same rate of salt removal in the range of 0.285 - 2.85 square meters of surface area. This figure is calculated from reported (143) values of 10^6 - 10^7 sq ft of membrane area required to produce 10 acre ft per hour of deionized water from sea water by the electrodialysis process.

To reduce the surface requirements to a practical level would require either a synthetic membrane or an organism with a surface highly more active than frog skin, or a synthetic membrane or organism with a great amount of surface area per unit volume. Since membrane structure very probably plays a highly important role in physiological transport, the possibility of developing a suitable synthetic membrane at the present elementary stage of knowledge on the subject is remote. It is possible, however, that some organisms may offset or reverse the area advantage of less than 1000 or 100 to 1 shown by the comparison of the type of synthetic membrane used in the electrodialysis process to frog skin. The alternative, utilizing an organism that offers a great amount of surface area per unit volume, would require the use of a microorganism. This is not only possible of attainment, but many industrial processes, such as sewage treatment and brewing, currently process large quantities of liquids by harnessing microorganisms.

B. Energy

Perhaps the most important yardstick to use in concluding whether consideration of physiological mechanisms should be included in

the saline water conversion program is that of energy requirements. Should a large advantage in energy efficiency be exhibited by physiological mechanisms over artificial mechanisms of separating salt and water, a definite interest in the former would be indicated. Even if no means to utilize the principles of physiological transport were apparent at present, fundamental research to explore the energy system for possible future application would be warranted. Based on limited data available in the literature, the following attempt is made to compare energy requirements of the physiological and artificial processes.

Data in reference 143 can be used to calculate the power requirements for the synthetic membrane electrodialysis process in deionizing sea water. This varies from 16,000 - 1,600 watt hr/gal in the reported range of surface areas discussed above. It has been reported (144) that optimum efficiencies that might be achieved by combinations of distillation processes could possibly distill water from sea water for approximately $100 per acre ft, power costs figured at four mills per kw hr. This would produce water at 76.6 watt hr/gal. The theoretical irreducible power requirement for extracting fresh water from sea water by any possible process has been calculated (145) at 2.8 watt hr/gal. This same reference cites the practical minimum figure that can conceivably be achieved by distillation or the electric membrane process as 12 watt hr/gal. In his work on frog skin, Zerahn (49) computed the energy requirement per transported equivalent of sodium to be 1,340 - 2,680 gm-cal. Assuming the chloride to follow passively, the power required to desalt one gallon of 30,000 ppm NaCl sea water in one hour may be calculated.

NaCl content of sea water = 113.5 gm/gal
This is 113.5/58.5 = 1.94 equivalents NaCl

Energy Required = 1.94 x 1,340 = 2,600 gm-cal
Which, if expended in one hour, would be
2,600 x .00116 = 3.015 watt hr/gal for the lower energy value reported and 2 x 3.015 = 6.030 watt hr/gal for the higher one.
Zerahn found the total energy available to frog skin to accomplish the transport of sodium to be a maximum of 5,000 gm-cal per equivalent. Thus, if the frog skin consumes the total available energy, the power expended per gallon of sea water becomes $\frac{5000}{2600}$ x 6.03 = 11.6 watt/hr.

It is quite interesting to note that these three values are below all of the practical minimum power requirements cited. The 3.015 watt hr/gal is surprisingly close to the 2.8 watt hr/gal theoretical minimum. Physiological processes thus seem to operate at efficiencies greater than those achieved or considered possible by mechanical, electrical or chemical processes of saline water conversion.

X. Evaluation of Practical Aspects

Certainly the state of knowledge does not permit the envisioning in the near future of an artificial application of the principles of physiological transport of sodium chloride. Therefore, any exploitation of physiological mechanisms at present would have to be approached through the use of living organisms or parts of organisms. As stated previously, living organisms are successfully used in many large-scale industrial processes. It would be logical and necessary to select the appropriate organisms on the basis of availability and ease of handling. A factor in the latter consideration would be the desirability of obtaining large active surface area per unit weight or volume. A group of organisms among which one or several might offer the required characteristics are the algae.

Some unicellular algae are known to accumulate sodium and chloride ions to concentrations manyfold that of their environment. The source of energy for the physiological transport of the ions, sunlight, is free. Unicellular algae can be grown in mass cultures (146), a process of much interest as a source of food supply. Since the surface area required per gal of sea water desalted per hr by frog skin is so large, 227 m^2, it might be well to investigate surface areas that may be available in algae on the basis that their ion transport mechanism may require comparably large surface areas per equivalent of sodium chloride transported. Algae suitable for the purpose at hand might possibly be found in the size range of chlorella. A steady-state culture of chlorella may contain approximately two cubic millimeters of cells per milliter of suspension. The cell diameter is approximately five microns. Assuming the cells to be

cubes, a simple calculation may be made giving the approximate cell surface area contained in one gallon of suspension.

surface area/cell = 150 microns^2

volume/cell = 125 microns^3

One ml of suspension contains $\frac{2 \times 10^9}{125} = 1.6 \times 10^7$ cells

This is $1.6 \times 10^7 \times 150 = 2.4 \times 10^9$ microns^2 of surface

$= 9.08 \times 10^{12}$ microns^2/gal suspension = 9.08 m^2.

and 227/9.08 = 25 gallons of suspension are required to offer sufficient surface to remove all the sodium chloride from 1 gal 30,000 ppm NaCl sea water in 1 hr.

Thus, if an alga approximately the size of chlorella were found to have the other necessary characteristics, the surface area requirements would be fairly easily satisfied. This would appear to be a possibility worth investigation.

In a truly practical sense, "practical" aspects should include fundamental research. There is obviously very much to be learned about salt transport as achieved in nature. Were this information known today, it is certain that it would either permit new and highly efficient methods of saline water conversion or greatly improve existing methods. Basic research may continue for a long time without producing an industrial process. However, the sooner the research is successfully accomplished, the sooner can its findings be applied to the mass production of fresh water.

XI. Design of Experimental Work

As has been indicated in the previous section, the quantities of salt to be removed from sea water, the rates at which salt can be transported physiologically, the surface area requirements thus determined, and the energies expended may allow a practical application of the physiological mechanism through the use of living organisms. The calculations upon which this finding is based assumed that the water being converted to zero salinity was sea water containing 30,000 ppm of sodium chloride. This, of course, is the extreme test. Should the salt load in sea water overtax the process, the method still might be useful in treating brackish waters. The fact that the calculations, approximate and incomplete as they no doubt are, do not preclude possible application to sea water presents a prospect worth exploring. It is unlikely that a definite answer to the possibility can be obtained by any theoretical treatment of the problem based on information currently available. The best and most direct means of exploring the subject lies in laboratory work. Only here can the paramount question of whether the algae can be made to behave as desired be settled.

The following suggested process for saline water conversion by the utilization of algae is believed to be novel and may warrant experimental consideration. Its application would require a search for marine algae having the most favorable characteristics as determined by rate and degree of sodium chloride concentrations, surface area, rapidity of response to the environment and adaptability for mass culturing.

The method would attempt to make algae behave as a sodium chloride "conveyor belt" by continually recycling them. This would greatly

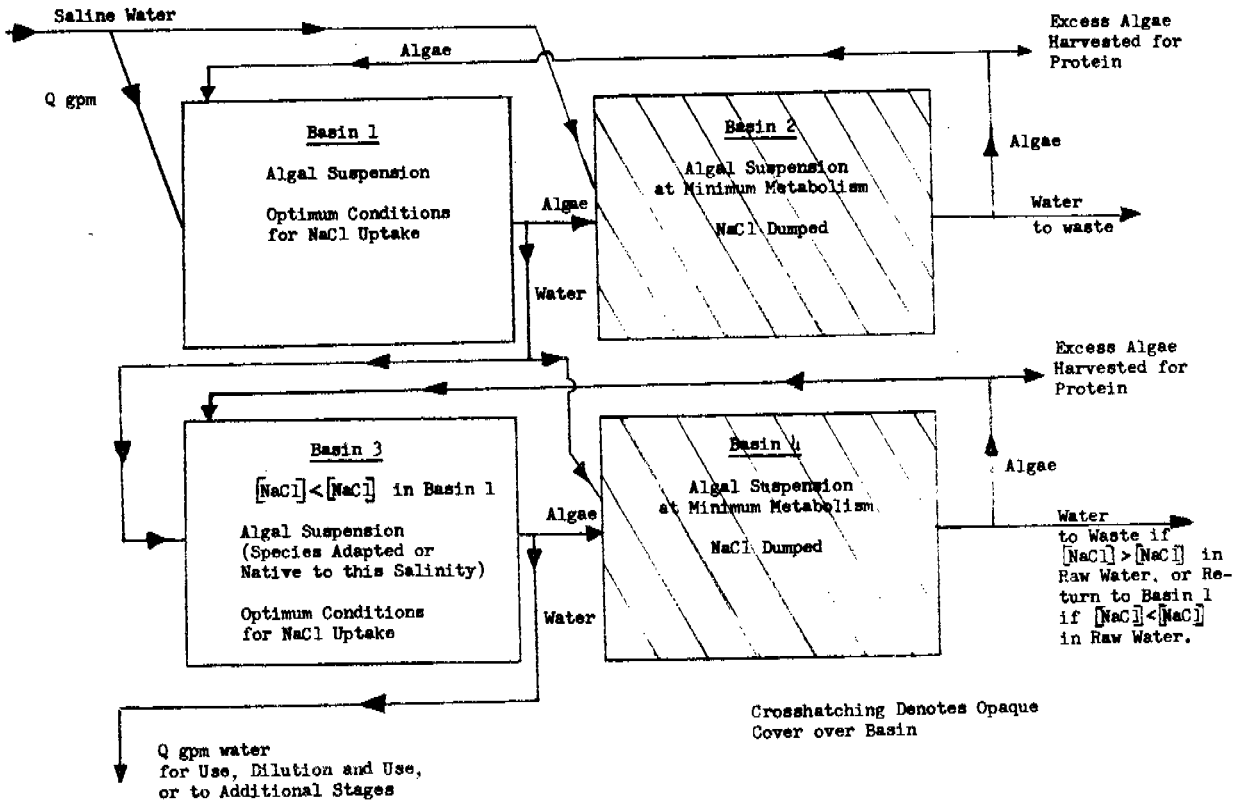
reduce the total quantity of cells required to be cultured per unit of sodium chloride absorbed as compared to a single-pass process. It would also speed up the process by eliminating the generation time which would otherwise be necessary to produce fresh algae. A species of algae with the desired characteristics might be grown in a basin of saline water under conditions to promote maximum incorporation of sodium chloride. The algae would be continuously removed from this basin by mechanical means and introduced into one end of a second basin of the water. It must now be remembered that, in order to maintain ion concentration gradients in physiological processes, metabolic energy must be expended. When cells die or are injured, the concentrations of ions inside the cells equilibrate with the concentrations of those ions in the surrounding aqueous environment. In the second basin, therefore, conditions would be established to deny the energy required by the algae for metabolism. This might be accomplished simply by excluding light from the basin. It is likely that the algae would then dump sodium chloride into the second basin. At the far end of this basin, the algae would be removed from the water and continuously transferred back to the first basin. Once again in a favorable environment, the cells would pick up another load of salt. While some algae can acclimate to a considerable range of sodium chloride concentrations, it may be necessary to operate several sets of basins in series with a different algal culture in each to reduce the sodium chloride content of the product water down to the desired level step by step. A by-product which might be of value would be the harvesting of excess algae for protein.

Figure 2 shows an hypothesized flow diagram for the process.

FIG. 2

Flow Diagram for Hypothesized Use of Algae in Saline Water Conversion

Showing Two-Stage Process



The basins are of flow-through design and of dimensions that will contain the required amount of process material and provide the necessary detention times. Flow is by gravity and the physiological energy is derived from sunlight. The only pecuniary energy input required is that expended mechanically in separating the algae from the suspension.

The saline water to be converted is introduced into basins 1 and 2. A mass algal culture of the appropriate type is seeded in basin 1 at the start of the process which from that point on becomes continuous. Conditions in basin 1 are maintained for optimum salt uptake by the algae. The velocity of flow through the basin allows the suspended algae sufficient time to absorb a maximum quantity of salt from the solution. At the effluent end of the basin, water is constantly taken into a device that separates the algae from it. The algae are delivered to basin 2 where conditions that will reversibly inhibit their metabolism are created. This would preferably be done by excluding light from the basin. At this point the sap of the algae is greatly enriched in sodium chloride as compared to its environment. To maintain this concentration gradient between its intercellular fluid and the suspending sea water should require the continuous expenditure of energy. The interruption of photosynthesis should deprive the cells of at least a substantial portion, if not all, of the needed energy. Accordingly, the algae should secrete sodium chloride as the concentration gradient approaches equilibrium. Transit through this basin would be timed to permit as much salt as possible to be dumped by the algae without inflicting irreversible damage to them. At the effluent end of basin 2 the suspension would again be mechanically separated into water and algae. The water, now higher in salt content than the original saline water, would be discharged to waste, which would

be back to the ocean in the event sea water was used as the source in the process. The algae would be returned to the influent side of basin 1, where their metabolism would be restored. Picking up another load of salt in traversing the basin, the algae would again be delivered to basin 2 to repeat the cycle. Thus, the water in basin 1 would become less saline. If the salinity were allowed to decline indefinitely, a point would be reached where the species of algae employed in basin 1 would no longer be able to tolerate the environment. Therefore at some predetermined point before this, the saline water influent would be regulated to obtain a balance between the quantity of salt introduced into the basin and the quantity removed. This would establish a dynamic equilibrium and maintain the mixed water in basin 1 at a fixed salt concentration less than that of the influent saline water.

The effluent water from basin 1, after having the algae removed, would be of this reduced salinity. It is unlikely, however, that any one species of algae can tolerate a sufficient range of salinity to permit sea water or very brackish water to be completely reclaimed in one stage. Therefore, several stages of the process using different species of algae, or algae of the same species adapted to different salinity ranges, would probably be required. For this reason, the basin 1 effluent would be delivered to basins 3 and 4. These latter basins would contain an algal culture compatible with a lower salinity range than that in basins 1 and 2, and the process described in basins 1 and 2 would be repeated. Thus

the effluent from basin 3 would be further reduced in salinity. Water for basin 4 must be drawn from basin 1. This is because the water in basin 4 would have to be lower in salinity than the raw water in order for the algae used in basins 3 and 4 to tolerate it. It is possible that at discharge the water in basin 4 would still be of less salinity than the raw water. In this event it would be returned to basin 1. However, if the salinity of the basin 4 effluent exceeded that of the raw water, the former would be wasted.

The effluent from basin 3 may require further salinity reduction before it could be used for irrigation, drinking or industrial purposes. If so, additional stages could be added to the process until the desired product would be achieved.

It will be noted that a portion of the product of each odd-numbered basin is required to operate the even-numbered basin of the succeeding stage. This product water, however, is merely stored in process. It is not lost. The effluent from each even-numbered basin would be returned to the lowest-numbered odd-numbered basin whose influent water was higher in salinity. As stated above, the possible exception might be basin 4 where the salinity of the effluent might be higher than that of the raw water entering basin 1. In this event, basin 4 effluent would be discharged to waste. Under steady state operation, the process would deliver desalted water at a rate equal, or nearly equal, to the rate at which raw saline water is introduced.

An additional feature of the process might be the production of algae as a valuable by-product. Since algae will multiply in the

odd-numbered basins, the excess may be harvested for protein. The harvest would probably be taken from the even-numbered basins so that the salinity of the algae would not make the product objectionable.

Part 2

LABORATORY EVALUATION OF USE OF ALGAE IN SALINE WATER CONVERSION
- PHASE I FINAL REPORT -

LABORATORY EVALUATION OF USE OF ALGAE IN SALINE WATER CONVERSION

PHASE I FINAL REPORT

I. Summary and Recommendations

The few species of algae previously known to concentrate sodium and chloride were found to have characteristics that rendered them undesirable for use in the conversion of saline waters by the method being evaluated. The emphasis of the project, therefore, was concentrated on a screening process to seek algae that did have favorable characteristics. A large number of species were tested for sodium and chloride uptake from saline media and for the resultant reduction in conductivity of the media. Many species were found to show good growth, be of the proper size range and concentrate sodium chloride. In a number of cases, the concentrating ability of the organisms was fairly high.

The following excerpts from Tables 4 and 5 are projected values based on the experimental data reported in Tables 2 and 3. In calculating these values it was assumed that fairly high mass culture densities could be maintained. While this assumption is favorable, it is possible of attainment and was used to obtain some indication of the potential of the algae.

Algae Demonstrating Good Uptake in 25 Percent Sea Water Medium

Species	Projected (pcv of 0.02 ml/ml assumed) Uptake from Original Medium (%)		
	Na^+	Cl^-	Cond.
Scenedesmus quadricauda	10	10	10
Chlamydomonas moewusii (696)	9	18	15
" simplex	13	7	9
" eugametos	20	14	15
Carteria species	10	10	5

Algae Demonstrating Good Uptake in 50 Percent Sea Water Medium

Species	Projected (pcv of 0.02 ml/ml assumed) Uptake from Original Medium (%)		
	Na^+	Cl^-	Cond.
Chlamydomonas intermedia	27	23	23
" pulchra	20	16	28
" species	17	17	17
" Species	30	20	20
Anabaena species	53	33	33
Anabaena catenula	30	30	23
Carteria chuii	17	20	17
Carteria species	10	20	10

Algae Demonstrating Good Uptake in 75 Percent Sea Water Medium

Species	Projected (pcv of 0.02 ml/ml assumed) Uptake from Original Medium (%)		
	Na^+	Cl^-	Cond.
Brachiomonas pulsifera	8	12	16

Algae Demonstrating Good Uptake in 100 Percent Sea Water Medium

Species	Projected (pcv of 0.02 ml/ml assumed) Uptake from Original Medium (%)		
	Na^+	Cl^-	Cond.
Monochrysis	none	30	40

There thus seems to be a good number of algae showing characteristics favorable to the proposed method of converting saline water. However, more caution should be exercised in dealing with the above projected values than with the experimental values. Any errors in the experimental values are likely to be compounded in the projected values. In addition, the routine mass culture of algae at densities of 0.02 milliliters of packed cells per milliliter of culture will not be easy to achieve. Nonetheless, the results are encouraging.

A disconcerting aspect of the data is that the final medium in a number of the uptake tests is reported to contain more sodium or chloride than at the beginning of the test. It is believed that this may be accounted for by errors in compensating for the volume changes undergone by the cultures during the test period. Secretions of some algae may possibly interfere with the flame determination of sodium as another source of this error.

The hardy and rapid growth of many species of fresh water algae in 25 and 50 percent sea water and of many of the marine algae in full-strength sea water is of considerable interest. It may be that algae can be grown for protein production in saline waters. This would be of great potential value to arid regions bordering bodies of salt water. It is possible that crop production might be obtained with no or minimal use of scarce fresh water.

The following specific recommendations are made:

1. While the results of the project do not permit the firm conclusion that the proposed method of saline water conversion is feasible,

the indications obtained are good. Accordingly, additional laboratory work should be carried out and the investigation continued.

2. Those algae showing favorable characteristics as determined by the currently reported work should be retested to confirm their uptake capabilities. Care should be taken to eliminate the above mentioned possible sources of error.

3. Additional algae should be screened for uptake.

4. Those algae presently reported as favorable and subsequently confirmed and new favorable species found should be studied for secretion of sodium and chloride. This would be done under conditions designed to inhibit metabolism of the algae as described by the proposed method of saline water conversion.

5. The possibility of growing marine or salt-tolerant species of algae in saline waters for protein production should be investigated.

II. Background

Part I consisted of a literature search in the fields of medicine, physiology, biochemistry and related disciplines. Ion transport in human and animal kidneys, intestinal mucosa, secretory glands, amphibian skin, fish, plants and individual cells was reviewed. These and all other living organic material constantly accumulate and excrete salts, including sodium chloride from aqueous solution. This is necessary in order to maintain vital electrolyte balances, On the basis of current information, there seems to be a striking similarity in physiological mechanisms that effect this action. Factors in common with many vari-

ties of animals, plants and cells are the abilities to accumulate and maintain ions against considerable concentration gradients through the expenditure of energy derived from metabolic processes. This process is universally performed at the cellular level. Although special organs, such as the kidney, may conserve ions within the organism, the ions must then be distributed throughout the cells. The intercellular fluid contains concentrations of selected ions at levels many times that of the extracellular fluid. In order to obtain and maintain this condition, the cell must expend metabolic energy at the cell wall.

The ability of isolated frog skin to transport sodium chloride from its outer to its inner side has been studied for many years. The action is typical of physiological transport in that the sodium and chloride ions are moved in a direction against the concentration gradient; that is, the ions are transported from the less saline outer solution to the more saline inner solution. Metabolic processes continue to supply energy for the process in the isolated skin for several hours. More data has been obtained about transport in frog skin than most, if not any, other organic materials. It has been reported (49) that the energy expended by frog skin is 1,340 - 2,680 gm-cal per equivalent of sodium transported. The chloride follows the sodium passively in order to maintain electrostatic balance. The power required to desalt one gallon of 30,000 ppm NaCl sea water in one hour may be calculated:

NaCl content of sea water = 113.5 gm/gal
This is 113.5/58.5 = 1.94 equivalents NaCl
Energy required = 1.94 x 1,340 = 2,600 gm-cal
Which, if expended in one hour, would be

2,600 x 0.00116 = 3.015 watt hr/gal for the lower energy value reported and 2 x 3.015 = 6.030 watt hr/gal for the higher one.

These energies are much closer to the theoretical minimum power requirement of 2.8 watt hr/gal of sea water desalted than are the energy requirements of any existing or proposed conversion process.

An average rate of sodium chloride transport through frog skin as determined from several references (49, 51, 54, 142, 147) is approximately 50×10^{-6} gm/hr/cm^2. The surface area required for complete desalting in one hour of one gallon of sea water containing 30,000 ppm sodium chloride can thus be calculated:

NaCl content of sea water = 113.5 gm/gal

$$\frac{113.5}{50 \times 10^{-6}} = 22.7 \times 10^5 \text{ cm}^2 = 227 \text{ m}^2 \text{ required}$$

While this is a prohibitively large surface area requirement for the use of any continuous surface in one plane, large surface areas per unit volume are available in finely divided material. Such finely divided organic material that conduct active transport of sodium and chloride are unicellular algae. Some unicellular algae are known (78) to accumulate sodium and chloride ions to concentrations considerably exceeding that of their environment. The source of energy for the physiological transport of the ions, sunlight, is free. Unicellular algae can be grown in mass cultures (146).

Since it has been found that ion transport processes in different organisms are quite similar, for the purposes of rough approximation it is assumed that the energy-surface ratio for transport in algae is of the order of that in frog skin. On this basis, the quantity of algae necessary to supply the required surface area may be estimated.

Algae suitable for the purpose at hand might possibly be found in the size range of chlorella. A steady state massive culture of chlorella may contain approximately two cubic millimeters of cells per milliliter of suspension. Assuming the cells to be cubes, a simple calculation may be made giving the approximate cell surface area contained in one gallon of suspension:

surface area/cell = 150 microns^2

volume/cell = 125 microns^3

One ml of suspension contains $\frac{2 \times 10^9}{125} = 1.6 \times 10^7$ cells

This is $1.6 \times 10^7 \times 150 = 2.4 \times 10^9$ microns^2 of surface

$= 9.08 \times 10^{12}$ microns^2/gal suspension $= 9.08$ m^2,

and 227/9.08 = 25 gallons of suspension are required to offer sufficient surface to remove all the sodium chloride from 1 gal 30,000 ppm NaCl sea water in 1 hour.

Thus, if an alga approximately the size of chlorella were found to have the necessary characteristics, the surface area requirements would be fairly easily satisfied.

Part I proposed a method of utilizing algae for the conversion of saline water. The method incorporated the unique feature of recycling the algae in order to reduce the quantity of algae that must be handled and to eliminate the generation time required for the production of fresh algae.

III. Objective

The objective of the work (Part I), was "to determine whether algae which are known to accumulate internal concentrations of salt in excess of the concentrations in their environment will secrete salt when their metabolism is inhibited." It soon developed, however, that, other than the several types of algae reported in the literature survey, no additional information was available concerning internal concentrations of sodium chloride accumulated by algae. It was also found that the types known to concentrate sodium chloride had size or culturing characteristics that made them unsuitable for the use intended.

It, thus, developed that an intensive screening program for suitable algae had to be instituted. This became the major effort of the research, since it was desired to accumulate as much information on this most important aspect of the suggested saline water conversion method in the brief span of the work. Accordingly, no tests were performed on salt secretion or on various ways to influence algal metabolism and sodium chloride uptake.

IV. Procedure

Fresh water and marine algal cultures were solicited from laboratory sources throughout the country. In all, something over 100 species were obtained and screened during the project. The cultures were maintained in cotton-stoppered, agar slant tubes of the stock culture medium. Composition of the stock culture medium was as follows:

Natural sea water (Atlantic)	950 ml
$NaNO_3$	1.0 gm
$NaH_2\ PO_4\ H_2O$	0.11 gm
Trihydroxymethylaminomethane	1.0 gm
Soil extract	50 ml
Agar	10 gm
HCl	to pH 7.0

The stock cultures were kept at 25° C under continuous fluorescent lighting of 200 foot candles intensity. Test media were prepared from Atlantic Ocean water taken off Ocean City, Maryland. The ingredients of various test media used are listed in Table 1. In essence, the media

TABLE 1

TEST MEDIA USED

Medium Designation	Composition		Na^+ (% of Medium C	Cl^- (ppm)	EC$\times 10^3$ (mhos)
A1	Medium C as diluted to 25% strength		25	4820	.372
A2	same		25	4820	.380
A3	same		25	5150	.395
A4	same		25	4900	.380
A5	same		25	5000	.385
A6	same		25	5150	.392
B1	Medium C as diluted to 50% strength		50	9260	.700
B2	same		50	9190	.698
B3	same		50	9300	.698
B4	same		50	9300	.705
B5	same		50	9230	.700
B6	same		50	9230	.283
C	Medium C		100	19250	.141
	Natural Sea Water (Atlantic Ocean)	1000 ml			
	$NaNO_3$	1.0 gm			
	$Na_2HPO_4 \cdot H_2O$	.11 gm			
	Trihydroxymethylaminomethane	1.5 gm			
	$Na_2SiO_3 \cdot 9H_2O$	40. mg			
	Na·EDTA*·Fe	40. mg			
	Na_2·EDTA*·Cu	7.8 mg			
	Na_2·EDTA*·Mn	21.4 mg			
	Na_2·EDTA*·Zn	6.8 mg			
	Na_2·EDTA*·Co	9.4 mg			
	Thiamine Chloride	0.2 mg			
	Biotin	1. ugm			
	Cobalamin (Vitamin B_{12})	1. ugm			
D	Medium C diluted to 25% and 5% soil extract added		25	4790	.370
E-1	Medium C diluted to 50% and 5% soil extract added		50	9340	.702
E-2	Medium C diluted to 50% and 5% soil extract added		50	9230	.685
F	Medium C diluted to 75% and 5% soil extract added		75	13300	.972

* EDTA - Ethylenediaminetetraacetate

consisted of full strength, 75 percent, 50 percent and 25 percent sea water with various nutrient and trace additives.

Medium C was adjusted to a pH of 8.1 with HCl where 5 percent CO_2-in-air was infused into it, and to a pH of 7.0 where air alone was infused. In preparation of the medium, the natural seawater, $NaNO_3$, NaH_2PO_4, trihydroxymethylaminomethane and HCl had to be mixed before the trace elements were added in order to prevent precipitation of the latter. The medium was then saturated with pure CO_2 to remove all traces of phosphate precipitates. The medium was sterilized by filtration for immediate use or stored in tightly stoppered, sterile bottles.

Soil extract was added to media D, E-1, E-2 and F. The extract was prepared by steaming equal weights of soil and tap water for two hours, allowing the suspended matter to settle and filtering the supernatant.

No attempt was made to determine the minimum number and concentration of additives required since the primary interest was focused on achieving rapid and good growth.

All uptake studies were conducted in test tube cultures maintained in transparent water baths at 25 $\pm$ 0.2° C, and were started at 650 foot candles of continuous, cool, white fluorescent light. When growth became clearly visible, the light intensity was increased to 1,000 foot candles. The cultures were aerated continuously with 5 percent CO_2-in-air or air alone.

Temperature of the water bath was maintained by a thermostatically controlled 200-watt immersion heater working against copper-cooling coils

supplied with a continuous flow of cold tap water. The water in the bath was continuously and vigorously mixed with electrical stirrers.

The water baths were made of clear lucite and were illuminated by parallel banks of 40-watt fluorescent lamps placed approximately four inches from the culture tubes. Six lamps were on each side of each bath and thus supplied equal intensity of light to both sides of the cultures.

Culture tubes were either 18-or 24-millimeter diameter Pyrex test tubes fitted with either cotton, in the case of pure cultures, or rubber plugs with central aeration tubes of 2-millimeter inside-diameter Pyrex tubing.

Aeration was supplied from a tank type air compressor and pure compressed CO_2. The mixture of CO_2-in-air was regulated by calibrated gas flowmeters. The gas was delivered to the culture tubes via brass distribution manifolds having 30 ports, each equipped with a needle valve. Delivery pressure was maintained in the range of 8-12 pounds per square inch.

Inocula for all experiments were prepared as follows:

1. A small inoculum was transferred from a stock culture to a test tube of the appropriate medium. The new culture was incubated on a reciprocating shaker with the culture tubes inclined at a 15° angle with the horizontal to permit thorough mixing. An illuminance of 700 foot candles of cool, white fluorescent light was maintained and the culture room was kept at 25°C.

2. When a culture density equivalent to 10 percent light transmittance, as measured through an 18-millimeter diameter cuvette, was attained, 10-milliliter aliquots of the test medium were inoculated with 0.25 milli-

liters each of the suspension. These cultures were used for the uptake tests. Care was taken to maintain the cultures in pure state up to the final step or throughout the entire procedure.

Each culture tested for uptake was sampled at two times during its development. The first sample was usually taken when the culture density approximated 10 percent transmission as judged visually. The second sample was usually taken approximately 48 hours later.

At each sampling, 3 milliliters of culture were withdrawn and centrifuged in a centrifuge tube having a specially designed, graduated capillary tip. After 15 minutes centrifugation at a field strength of 1,100 times that of gravity, the packed volume became stabilized and was read directly in the graduated capillary. Then, exactly 2 milliliters of cell-free medium was transferred to a Neutraglass sample bottle containing 98 milliliters of glass-distilled water. The contents were thoroughly shaken and were used for the uptake analyses.

The following analyses were made:

1. Sodium. Sodium was determined by standard flame spectrophotometric analysis using the Beckman Model DU equipped with a hydrogen-oxygen flame attachment. Slit width was 0.025 millimeters and a wave length of 589 millimicrons was used. The ratio of oxygen-to-hydrogen gas pressures was 10 pounds per square inch to 4.5 pounds per square inch. Each sample was compared with an equivalent sample of unused medium. Curves relating luminosity to sodium concentrations were prepared for the range under investigation. Sodium in the final medium was determined as percent of sodium in the original medium.

2. Conductivity. Conductivity was measured with a General Industries 0.2 to 2,000,000-ohm electronic bridge with a 15-milliliter, pipette-type conductivity cell. All readings were corrected to 25°C. The cell constant with 0.1N KCl was 0.328. Conductivities of the medium before and after growth had taken place in it were determined and compared.

3. Chloride. Chloride content was established by conventional $AgNO_3$ titration conducted with a chromate indicator. The strength of the $AgNO_3$ solution was adjusted to require at least 5 milliliters per titration. KCl was used to standardize the $AgNO_3$ solutions.

Duplicate cultures were always carried throughout the course of the analyses made on each. Triplicate sodium analyses were made on each sample and the average value used. Duplicate conductivity and chloride analyses were made and averaged for each sample.

V. Results

The results obtained from the uptake studies on fresh water algae are presented in Table 2. The same data for marine algae are given in Table 3. Only those algae which showed growth are listed in these tables. The code designation of the media refer to Table 1 which gives the compositions of the media. The percent sea water is given again in Table 2, however, for ready reference. The number of hours which the organisms grew prior to uptake determinations and the density attained, in terms of milliliters of packed cell volume per milliliter of culture, are listed. Generally there are two sets of determinations for each alga as uptake tests were made at two points of growth. The sodium and chloride content and the

TABLE 2

EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after Growth (All as % of medium before inoculation)			AFTER GROWTH Intracellular* / Final Medium	
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Chlamydomonas species	A-1	25	72	.002	lost	lost	lost	-	-
" "	A-1	25	120	.017	103	104	101	-	-
Stichococcus bacillaris	A-1	25	146	.006	103	101	101	-	-
" "	A-1	25	170	.010	104	99	101	-	-
Scenedesmus obliquus	A-1	25	96	.007	lost	lost	lost	-	-
" "	A-1	25	144	.021	106	104	103	-	-
Chlorella vulgaris	A-1	25	72	.002	lost	lost	lost	-	-
" "	A-1	25	120	.016	103	100	98	-	-
Chlamydomonas reinhartii	A-1	25	72	.012	lost	lost	lost	-	-
" "	A-1	25	120	.025	101	100	98	-	-
Scenedesmus quadricauda	A-1	25	72	.004	98	98	98	5.1	5.1
" "	A-1	25	120	.021	99	99	97	2.1	2.1
Chlorella vulgaris	A-1	25	72	.003	101	100	102	-	-

* Reported only where uptake took place

TABLE 2 (continued)

EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after Growth (All as % of medium before inoculation			AFTER GROWTH Intracellular Final Medium	
	Medium	%Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Chlorella vulgaris	A-1	25	120	.018	105	104	101	-	-
Scenedesmus basilensis	A-2	25	77	.003	99	101	98	3.3	-
" "	A-2	25	125	.011	101	98	95	-	1.9
Coelastrum proboscideum	A-2	25	149	.006	99	92	96	1.7	14.4
Protosiphon botryoides	A-2	25	77	.004	96	101	100	2.5	-
" "	A-2	25	125	.015	100	99	lost	-	0.7
Gyroffiana humicola	A-3	25	115	.003	102	99	101	-	3.8
" "	A-3	25	160	.010	100	97	101	-	3.1
Oocystis Naegelii	A-3	25	168	.007	98	95	96	2.9	3.0
" "	A-3	25	184	.013	97	99	90	2.3	0.8
Chlamydomonas Moewusii (mutant 697)	A-3	25	72	.004	102	99	100	-	2.5
" " "	A-3	25	115	.016	104	94	96	-	3.9
Chlamydomonas inflexa	A-3	25	72	.003	103	100	104	-	-

* Reported only where uptake took place

TABLE 2 (continued)
EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after Growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular*	
								Final	Medium
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Chlamydomonas inflexa	A-3	25	160	.017	104	91	99	-	5.7
Chlorella ellipsoidea	A-3	25	72	.002	103	103	104	-	-
" "	A-3	25	120	.014	103	97	96	-	2.2
Chlamydomonas incerta	A-3	25	72	.007	103	100	103	-	-
" "	A-3	25	120	.016	97	95	95	1.9	3.2
Chlamydomonas moewusii (mutant 696)	A-3	25	168	.007	99	90	93	1.5	15.8
" "	A-3	25	240	.009	96	92	93	4.6	9.6
Palmellococcus miniatus	A-4	25	48	.003	103	99	99	-	3.4
" "	A-4	25	84	.016	108	102	101	-	-
Chlorella pyrenoidosa (Van Niel)	A-4	25	48	.001	104	100	100	-	-
" " "	A-4	25	84	.015	108	104	100	-	-
Chlorella pyrenoidosa (Emerson)	A-4	25	48	.002	102	94	97	-	31.7
" " "	A-4	25	84	.020	105	98	99	-	-

* Reported only where uptake took place

TABLE 2 (continued)
EXPERIMENTAL RESULTS
Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after Growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular* Final	Medium
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Chlamydomonas pseudococcum	A-4	25	84	.015	106	100	100	-	-
" "	A-4	25	108	.020	101	99	97	-	-
Scenedesmus species	A-4	25	90	.009	103	99	97	-	1.1
" "	A-4	25	120	.017	101	96	96	-	2.4
Ankistrodesmus braunii	A-4	25	85	.004	105	99	100	-	2.5
Chlamydomonas simplex	A-5	25	120	.011	93	96	95	6.7	3.7
Chlamydomonas intermedia	A-5	25	72	.005	102	104	103	-	-
" "	A-5	25	96	.016	98	97	95	1.3	1.9
Chlamydomonas species	A-5	25	72	.005	101	103	102	-	-
" "	A-5	25	96	.011	96	92	92	3.7	7.8
Chlamydomonas pulchra	A-5	25	72	.003	101	99	104	-	3.4
" "	A-5	25	96	.015	94	94	93	4.2	4.2
Chlamydomonas eugametes	A-5	25	90	.009	97	103	98	3.4	-

*Reported only where uptake took place

TABLE 2 (continued)
EXPERIMENTAL RESULTS
Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after Growth (All as % of medium before inoculation			AFTER GROWTH	
								Intracellular*	
				Final Density				Final	Medium
	Medium	% Sea Water	Hours	PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Chlamydomonas eugametos	A-5	25	110	.013	89	91	90	9.3	7.5
Chlamydomonas moewusii	A-5	25	66	.003	100	101	100	-	-
" "	A-5	25	96	.010	98	99	98	2.1	-
Chlamydomonas gyrus	A-5	25	72	.009	100	103	104	-	-
" "	A-5	25	90	.013	99	95	99	-	4.0
Chlamydomonas species	B-1	50	96	.05	102	103	103	-	-
" "	B-1	50	146	.011	102	103	101	-	-
Stichococcus pacillaris	B-1	50	170	.004	102	101	104	-	-
Scendesmus obliquus	B-1	50	96	.002	100	100	99	-	-
" "	B-1	50	144	.007	102	97	97	-	4.4
Chlorella vulgaris	B-1	50	120	.006	107	102	101	-	-
" "	B-1	50	168	.013	104	97	100	-	2.3

* Reported only when uptake took place

TABLE 2 (continued)
EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after Growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular* Final Medium	
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Chlamydomonas reinnartii	B-1	50	96	.004	100	100	100	-	-
" "	B-1	50	168	.014	96	94	97	2.9	4.4
Scenedesmus quadricauda	B-1	50	72	.003	101	100	98	-	-
" "	B-1	50	120	.019	104	104	100	-	-
Chlorella vulgaris	B-1	50	96	.004	100	100	100	-	-
" "	B-1	50	144	.019	101	103	100	-	-
Scenedesmus basilensis	B-2	50	120	Trace	-	-	-	-	-
Coelastrum proboscideum	B-2	50	132	Trace	-	-	-	-	-
Protosciphon botrycides	B-2	50	77	.004	100	99	101	-	2.5
" "	B-2	50	125	.013	101	101	99	-	-
Gyroffiana humicola	B-3	50	115	.002	103	102	102	-	-
" "	B-3	50	160	.003	108	100	98	-	-

* Reported only where uptake took place

TABLE 2 (continued)
EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after Growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular* Final Medium	
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Oocystis Naegelii	B-3	50	168	.005	101	100	102	-	-
" "	B-3	50	184	.011	105	101	101	-	-
Chlamydomonas moewusii (mutant 697)	B-3	50	115	.007	102	97	100	-	4.4
" " "	B-3	50	160	.015	105	101	97	-	-
Chlamydomonas inflexa	B-3	50	160	.006	99	97	96	1.7	5.1
" "	B-3	50	208	.010	96	101	96	4.1	-
Chlorella ellipsoidea	B-3	50	120	.006	101	99	98	-	1.7
" "	B-3	50	160	.017	107	100	100	-	-
Chlamydomonas incerta	B-3	50	72	.005	103	105	101	-	-
" "	B-3	50	120	.016	100	99	97	-	-
Chlamydomonas moewusii (mutant 696)	B-3	50	168	.003	103	96	98	-	13.8
" " "	B-3	50	240	.009	105	98	99	-	2.3

* Reported only where uptake took place

TABLE 2 (continued)
EXPERIMENTAL RESULTS
Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after Growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular*	
								Final	Medium
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Palmellococcus miniatus	B-4	50	48	.002	101	98	99	-	10.2
" "	B-4	50	84	.014	109	102	101	-	-
Chlorella pyrenoidosa (Van Niel)	B-4	50	48	.001	102	101	102	-	-
" " "	B-4	50	84	.014	109	105	103	-	-
Chlorella pyrenoidoso (Emerson)	B-4	50	48	.002	102	101	96	-	-
" " "	B-4	50	84	.013	103	100	99	-	-
Chlamydomonas pseudococcum	B-4	50	84	.007	105	97	100	-	4.4
" "	B-4	50	108	.010	102	99	98	-	-
Scenedesmus species	B-4	50	90	.003	105	102	99	-	-
Coccomyxa simplex	B-4	50	168	Trace	-	-	-	-	-
Ankistrodesmus braunii	B-4	50	165	Trace	-	-	-	-	-
Chlamydomonas simplex	B-5	50	120	.006	94	98	97	6.9	3.3
Chlamydomonas intermedia	B-5	50	96	.006	92	93	93	14.4	12.5
" "	B-5	50	120	.011	95	95	95	4.7	4.7

*Reported only where uptake took place

TABLE 2 (continued)
Experimental Results

Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular* Final Medium	
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Chlamydomonas species	B-5	50	96	.006	95	95	95	6.7	6.7
" "	B-5	50	120	.012	96	96	96	3.4	3.4
Chlamydomonas pulchra	B-5	50	96	.005	95	96	93	10.5	12.5
" "	B-5	50	120	.012	96	98	94	3.4	1.7
Chlamydomonas eugcmetos	B-5	50	90	.009	99	104	100	1.1	-
" "	B-5	50	110	.013	94	96	94	4.7	3.2
Chlamydomonas moewusii	B-5	50	120	.008	95	102	99	6.6	—
Chlamydomonas gyrus	B-5	50	72	.003	101	105	104	-	-
" "	B-5	50	90	.008	97	103	99	3.8	-
Anabaena species	D	25	240	.008	98	101	132	2.6	-
" "	D	25	288	.012	90	100	98	9.1	—
Anacystis midulans	D	25	144	.009	98	99	99	2.2	1.1

* Reported only where uptake took place

TABLE 2 (continued)
EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular* Final Medium	
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Anacystis midulans	D	25	166	.010	99	105	101	-	-
Euglena gracilis	D	25	144	.021	101	100	98	-	-
" "	D	25	166	.027	97	99	98	1.1	-
Anabaena catenula	D	25	240	.005	102	104	104	-	-
" "	D	25	288	.021	87	101	97	6.3	-
Anabaena cylindrica	D	25	240	.007	98	99	99	2.9	1.4
" "	D	25	312	.012	99	105	101	-	-
Anabaena species	E-1	50	240	.002	103	112	111	-	-
" "	E-1	50	288	.003	92	95	95	2.9	1.8
Anabaena variabilis	E-1	50	240	.002	105	103	103	-	-
" "	E-1	50	288	.007	102	99	99	-	1.4
Anacystis midulans	E-1	50	144	.005	100	94	93	-	12.7

* Reported only where uptake took place

TABLE 2 (continued)
EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium Before Inoculation		Growth		Medium after growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular*	
								Final	Medium
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Anacystis midulans	E-1	50	166	.009	98	103	102	2.2	-
Euglena gracilis	E-1	50	144	.005	101	94	85	-	12.7
" "	E-1	50	166	.012	103	99	98	-	-
Anabaena catenula	E-1	50	240	.002	102	109	110	-	-
" "	E-1	50	288	.006	91	91	93	16.5	16.5
Anabaena cylindrica	E-1	50	240	.007	100	94	93	-	9.0
" "	E-1	50	312	.010	98	103	102	2.0	-

* Reported only where uptake took place

TABLE 3

EXPERIMENTAL RESULTS

Marine Algae

Species	Medium before Inoculation		Growth		Medium After Growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular* Final Medium	
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Chlamydomonas species	A-2	25	53	.002	97	100	100	15.5	-
" "	A-2	25	96	.021	100	99	94	-	-
Nannochloris atomus	A-2	25	77	.003	101	100	106	-	-
" "	A-2	25	125	.026	101	100	94	-	-
Porphyridium species	A-2	25	77	.005	100	100	99	-	-
P-141[2]	A-6	25	102	.007	102	99	97	-	1.4
"	A-6	25	126	.013	100	107	102	-	-
Stichococcus basillaris[1]	A-6	25	126	.001	103	107	108	-	-
" "	A-6	25	198	.007	103	93	98	-	10.7
Chlorella species	A-6	25	78	.009	97	92	98	3.4	9.6
" "	A-6	25	102	.019	99	106	102	-	-

[1] Normally considered to be fresh water variety, but obtained here from marine culture.
[2] Unidentified.
* Reported only where uptake took place.

TABLE 3

EXPERIMENTAL RESULTS

Marine Algae

Species	Medium before Inoculation		Growth		Medium After Growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular* Final Medium	
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Carteria chuii	A-6	25	126	.004	99	104	103	2.5	-
" "	A-6	25	198	.017	97	99	95	1.8	1.2
Carteria species	A-6	25	78	[illegible]	100	98	96	-	10.2
" "	A-6	25	102	[illegible]	96	96	99	5.2	5.2
Platymonas subcordiformis	A-6	25	102	.003	102	95	97	-	17.6
" "	A-6	25	186	[illegible]	100	102	103	-	-
Chlamydomonas species	B-2	50	53	[illegible]	7	98	97	[illegible]	10.2
" "	B-2	50	96	[illegible]	[illegible]	98	99	-	-
Nannochloris otomus	B-2	50	72	[illegible]	98	100	98	[illegible]	-
" "	B-2	50	120	.025	101	101	99	-	-
Porphyridium species	B-2	50	77	.007	100	100	101	-	-

* Reported only where uptake took place.

TABLE 3 (continued)

EXPERIMENTAL RESULTS

Marine Algae

Species	Medium before Inoculation		Growth		Medium After Growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular* Final	Medium
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
P-141[2]	B-6	50	126	.007	101	105	102	-	-
"	B-6	50	198	.018	98	98	100	1.1	1.1
Stichococcus basillaris[1]	B-6	50	198	.003	94	102	102	21.2	-
Chlorella species	B-6	50	78	.009	97	92	98	3.3	9.6
" "	B-6	50	102	.019	99	106	102	1.4	-
Carteria chuii	B-6	50	78	.002	94	101	95	31.5	-
" "	B-6	50	102	.008	93	92	93	9.4	10.8
Carteria species	B-6	50	78	.004	98	96	98	5.1	10.6
" "	B-6	50	102	.012	97	97	96	2.5	2.5
Platymonas subcordiformis	B-6	50	78	.004	99	98	99	2.5	5.1
" "	B-6	50	102	.011	97	95	97	2.8	4.7

[1] Normally considered to be fresh water variety, but obtained here from marine culture.
[2] Unidentified.

* Reported only where uptake took place.

TABLE 3 (continued)
EXPERIMENTAL RESULTS

Marine Algae

Species	Medium before Inoculation		Growth		Medium After Growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular* Final	Medium
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Dunaliella salina	B-6	50	198	.003	90	90	103	36.7	36.7
Dunaliella euchlora	C	100	116	.021	100	101	100	-	-
" "	C	100	140	.025	97	95	95	1.2	2.1
Monochrysis	C	100	116	Trace	100	100	102	-	-
"	C	100	168	.002	99	93	94	5.0	37.5
Dunaliella salina	C	100	140	.009	99	97	99	1.1	3.4
" "	C	100	170	.020	98	96	97	-	5.1
Chlorella species (Lewin)	C	100	140	.010	100	99	99	-	-
" " "	C	100	164	.015	100	96	96	-	2.7
Brachiomonas submarina	E-2	50	120	.008	99	101	100	1.2	-
Brachiomonas pulsifera	E-2	50	96	.004	100	101	99	-	-
" "	E-2	50	120	.010	102	102	103	-	-

* Reported only where uptake took place.

TABLE 3 (continued)
EXPERIMENTAL RESULTS

Marine Algae

Species	Medium Before Inoculation		Growth		Medium After Growth (All as % of medium before inoculation)			AFTER GROWTH	
								Intracellular* Final Medium	
	Medium	% Sea Water	Hours	Final Density PCV (ml/ml)	Na^+	Cl^-	Cond.	Na^+	Cl^-
Cryptomonas species	E-2	50	96	.006	102	99	99	-	1.7
" "	E-2	50	120	.011	101	102	101	-	-
Brachiomonas submarina	F	75	120	.002	98	101	100	10.2	-
Brachiomonas pulsifera	F	75	96	.005	98	97	96	4.1	6.2
" "	F	75	120	.012	100	101	100	-	-
Cryptomonas species	F	75	96	.005	100	101	100	-	-
" "	F	75	120	.007	101	103	102	-	-

* Reported only where uptake took place

conductivity of the media after growth of the algae are given as percentages of these values in the initial media. The last two columns of the table present the ratios of the intracellular concentrations of sodium and chloride to the concentrations of these ions in the medium after growth has occurred. They show the ability demonstrated by various algae to concentrate sodium or chloride above the concentrations of these ions in the environment of the organisms. For example, the figure 5.1 in the column headed "After Growth, Intracellular/ Final Medium, Na" means that the alga cited concentrated sodium to a factor of 5.1 times the sodium concentration in the medium at the time the determination was made. This information is presented only where the internal concentrations exceeded the external ones.

VI. Discussion of Results

Uptake analyses were made on 36 fresh water species and 16 marine species. No analyses were made on many other species screened since they did not grow in the screening media. Almost all of the fresh water algae were tested in 25 and 50 percent sea water media. Most of the marine algae were also tested in 25 and 50 percent sea water media. Some of the latter were tested in 75 and 100 percent sea water media.

Twenty-seven of the fresh water species grown in 25 percent sea water medium demonstrated a reduction of two percent or more in the sodium or chloride content or conductivity of the medium. Of these, 20 reduced one or more of these characteristics by four percent or more. Eleven of this latter group achieved reductions of six percent or greater. Eight of them

reduced one or more of these three indices by eight percent or more, and four of these achieved reductions of 10 percent or more. Twenty-five fresh water species grown in 50 percent sea water demonstrated reductions of two percent or more in one or more of the three quantities mentioned above. Of these, 14 reduced one or more of the three indices by four percent or more. Nine of these achieved reductions of six percent or more. Six of the latter reduced one or more of the medium characteristics measured by eight percent or more, and one of these achieved a reduction of more than 10 percent.

Eight marine species grown in 25 percent sea water medium demonstrated reductions of two percent or more in one or more of the above three indices of the medium. These reductions equaled or exceeded four percent in six of these species. Five of them showed reductions of six or more percent. Two showed reductions of eight or more percent, one of which exceeded 10 percent.

Nine marine species grown in 50 percent sea water medium showed similar reductions of two percent or more. Six of these equaled or exceeded four percent reductions. Four of the latter showed reductions of six percent or greater. Three of these were in the range of eight percent or greater and one of the latter equaled 10 percent.

One marine species grown in 75 percent sea water medium showed reductions in medium content of sodium, chloride and in conductivity of two, three and four percents, respectively.

Three marine species grown in full strength sea water demonstrated uptakes in one or more of the medium indices of four percent or more,

one of which showed a reduction in chloride of seven percent and a reduction in conductivity of six percent.

All of the reductions of sodium, chloride and conductivity of the various media were those achieved by the organisms in the culture densities to which they had grown at the time the tests were made. Since these varied widely, an attempt was made to show the effects demonstrated by the algae in terms of internal to external concentration of sodium and chloride and of conductivity. These values, calculated, of course, only for those algae which achieved uptake, are shown in the last two columns in Tables 2 and 3. These values vary quite widely for the various species. Many are in the order of five-to-10-fold concentrations. Several range in the vicinity of 30-to 40-fold concentrations.

The achievements of many of the algae seem quite significant. However, there is one particular aspect of the results which indicates that firm conclusions should not yet be drawn. This is the fact that approximately 25 percent of the sodium determinations and 20 percent of the chloride determinations indicated that the concentrations of these ions in the test media had increased by two or more percent during the time the algae grew. This, of course, is impossible. Algae cannot manufacture sodium or chloride and the initial inocula were too small to contain these quantities of the two ions so that they could not have been secreted by the algae. It is possible, however, that certain metabolic products secreted by the algae could have interfered with the flame determination of sodium. No such explanation seems likely for chloride. The most logical explanation for these variances seems to lie in the culture volume corrections which were required to calculate the results on the basis of constant volume. Without

these corrections, it would have seemed that the algae had taken in considerable quantities of ions when humidity conditions were such that the aeration process deposited moisture in the culture to increase its volume. Conversely, if the volume had been decreased by evaporation, the apparent concentration of ions would have increased at the end of the experiment. The correction factors were carefully applied on the basis of referral to the original meniscus line which was marked on the tube. The factor which implicates this correction as being responsible in greatest part for the terminal values of sodium and chloride sometimes exceeding 100 percent of the original values is that the percent changes in sodium and chloride compared closely within each culture tube, but, on these occasions, varied between the culture tube replicates. The duplicate analyses made on each sample were also in good agreement, indicating that the analytical methods and techniques were satisfactory.

An attempt was made to project the experimental results to a common culture density to compare the magnitudes of uptake that might possibly be achieved by the various algae showing some promise in saline water conversion. The density selected for the projection, a packed cell volume of 0.02 milliliters of cells per milliliter of culture, is somewhat high for mass cultures, but may possibly be attained. Several of the test cultures actually exceeded this density. A value in the high range was selected to provide a gage against which the ultimate feasibility of the use of algae in converting saline water could be measured. Accordingly, Tables 4 and 5 were constructed by converting the experimentally obtained uptake values for sodium, chloride and conductivity to values that would have been obtained if the cultures had contained 0.02 milliliters of packed cells per milliliter or culture. This, of course, assumes that the metabolism of the individual alga would remain

TABLE 4

PROJECTED UPTAKES BASED ON EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium		Final Density PCV (ml/ml)	Measured uptake from medium (as % of original medium)			Calculated uptake from medium based on assumed pcv of .00 ml/ml (as % of original medium)*		
	Code	% Sea Water		Na^+	Cl^-	Cond.	Na^+	Cl^-	Cond.
Chlorella vulgaris	A-1	25	.016	-	-	2	-	-	2
Scenedesmus quadricauda	A-1	25	.004	2	2	2	20	20	10
"	A-1	25	.021	-	-	3	-	-	2
Scenedesmus basilensis	A-2	25	.003	-	-	4	-	-	15
"	A-2	25	.011	-	2	5	-	4	-
Coelastrum proboscideum	A-2	25	.006	-	3	1	-	26	13
Protosiphon botyroides	A-2	25	.004	4	-	-	10	-	-
Gyroffiana humicola	A-3	25	.010	-	3	-	-	6	-
Oocystis Naegelii	A-3	25	.007	2	5	4	6	13	11
"	A-3	25	.013	3	-	10	5	-	15
Chlamydomonas Moewusii (mutant 697)	A-3	25	.016	-	6	4	-	7	5
Chlamydomonas inflexa	A-3	25	.017	-	9	-	-	11	-
Chlorella ellipsoidea	A-3	25	.014	-	2	4	-	14	6
Chlamydomonas incerta	A-3	25	.016	3	5	5	4	6	6
Chlamydomonas moewusii (mutant 696)	A-3	25	.007	-	10	7	-	28	20
"	A-3	25	.009	4	8	7	9	18	15
Chlorella pyrenoidosa (Emerson)	A-4	25	.002	-	6	3	-	60	30
"	A-4	25	.020	-	2	-	-	2	-

* Reported only where uptake took place.

TABLE 4 (continued)
PROJECTED UPTAKES BASED ON EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium		Final Density PCV (ml/ml)	Measured uptake from medium (as % of original medium)*			Calculated uptake from medium based on assumed pcv of .02 ml/ml (as % of original medium)*		
	Code	% sea water		Na^+	Cl^-	Cond.	Na^+	Cl^-	Cond.
Chlamydomonas pseudococcum	A-4	25	.02	-	-	3	-	-	3
Scenedesmus species	A-4	25	.009	-	-	3	-	-	7
"	A-4	25	.013	-	4	4	-	5	5
Chlamydomonas simplex	A-5	25	.011	7	4	5	13	7	9
Chlamydomonas intermedia	A-5	25	.016	2	3	5	2	4	6
Chlamydomonas species	A-5	25	.011	4	8	8	7	5	5
Chlamydomonas pulchra	A-5	25	.015	6	6	7	8	8	9
Chlamydomonas eugametos	A-5	25	.009	3	-	2	7	-	4
"	A-5	25	.013	11	9	10	20	14	15
Chlamydomonas moewusii	A-5	25	.010	2	-	2	4	-	4
Chlamydomonas gyrus	A-5	25	.013	-	5	-	-	8	-
Scenedesmus obliquus	B-1	50	.007	-	3	3	-	8	8
Chlorella vulgaris	B-1	50	.013	-	3	-	-	5	-
Chlamydomonas reinhartii	B-1	50	.014	4	6	3	6	9	4
Scenedesmus quadricauda	B-1	50	.003	-	-	2	-	-	13
Gyroffiana humicola	B-3	50	.003	-	-	2	-	-	13
Chlamydomonas moewusii (strain 697)	B-3	50	.007	-	3	-	-	30	-
"	B-3	50	.015	-	-	3	-	-	4

* Reported only where uptake took place.

TABLE 4 (continued)

PROJECTED UPTAKES BASED ON EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium		Final Density PCV (ml/ml)	Measured uptake from medium (as % of original medium)*			Calculated uptake from medium based on assumed pcv of .02 ml/ml (as % of original medium)*		
	Code	% sea water		Na^+	Cl^-	Cond.	Na^+	Cl^-	Cond.
Chlamydomonas inflexa	B-3	50	.006	-	3	4	-	10	13
"	B-3	50	.010	4	-	2	2	-	8
Chlorella ellipsoidea	B-3	50	.006	-	-	2	-	-	7
Chlorella incerta	B-3	50	.016	-	-	1	-	-	4
Chlamydomonas moewusii (strain 696)	B-3	50	.003	-	4	8	-	27	13
"	B-3	50	.009	-	2	-	-	4	-
Palmellococcus miniatus	B-4	50	.002	-	2	-	-	20	-
Chlorella pyrenoidosa (Emerson)	B-4	50	.002	-	-	4	-	-	40
Chlamydomonas pseudococcum	B-4	50	.007	-	3	-	-	8	-
"	B-4	50	.010	-	-	2	-	-	4
Chlamydomonas simplex	B-5	50	.006	6	2	3	20	7	10
Chlamydomonas intermedia	B-5	50	.006	8	7	7	27	23	23
"	B-5	50	.011	5	5	5	9	9	9
Chlamydomonas species	B-5	50	.006	5	5	5	17	17	17
"	B-5	50	.012	4	4	4	7	7	7
Chlamydomonas pulchra	B-5	50	.005	5	4	7	20	16	28
"	B-5	50	.012	4	2	6	7	3	10
Chlamydomonas eugometos	B-5	50	.013	6	4	6	9	6	9
Chlamydomonas moewusii	B-5	50	.008	5	-	-	12	-	-

* Reported only where uptake took place.

TABLE 4 (continued)

PROJECTED UPTAKES BASED ON EXPERIMENTAL RESULTS

Fresh Water Algae

Species	Medium		Final Density PCV (ml/ml)	Measured uptake from medium (as % of original medium)*			Calculated uptake from medium based on assumed pcv of .02 ml/ml (as % of original medium)*		
	Code	% Sea Water		Na^+	Cl^-	Cond.	Na^+	Cl^-	Cond.
Chlamydomonas gyrus	B-5	50	.008	3	-	-	7	-	-
Anabaena species	D	25	.008	2	-	-	5	-	-
"	D	25	.012	10	-	2	16	-	3
Anacystis midulans	D	25	.009	2	-	-	4	-	-
Anabaena catenula	D	25	.021	13	-	3	12	-	3
Anabaena cylindrica	D	25	.007	2	-	-	6	-	-
Anabaena species	E-1	50	.003	8	5	5	53	33	33
Anacystis midulans	E-1	50	.005	-	6	7	-	24	28
"	E-1	50	.009	2	-	-	4	-	-
Euglena gracilis	E-1	50	.005	-	6	15	-	24	60
"	E-1	50	.012	-	-	2	-	-	4
Anabaena catenula	E-1	50	.006	9	9	7	30	30	23
Anabaena cylindrica	E-1	50	.007	-	6	7	-	17	20
"	E-1	50	.010	2	-	-	4	-	-

* Reported only where uptake took place.

TABLE 5

PROJECTED UPTAKES BASED ON EXPERIMENTAL RESULTS

Marine Algae

Species	Medium		Final Density PCV (ml/ml)	Measured uptake from medium (as % of original medium)*			Calculated uptake from medium based on assumed pcv of .02 ml/ml (as % of original medium)*		
	Code	% Sea Water		Na^+	Cl^-	Cond.	Na^+	Cl^-	Cond.
Chlamydomonas species	A-2	25	.002	3	-	-	30	-	-
"	A-2	25	.021	-	-	6	-	-	6
Nannochloris atomus	A-2	25	.026	-	-	6	-	-	5
P-141[2]	A-6	25	.007	-	-	3	-	-	8
Stichococcus basillaris[1]	A-6	25	.007	-	7	2	-	20	6
Chlorella species	A-6	25	.009	3	8	2	7	18	4
Carteria chuii	A-6	25	.017	3	-	5	3	-	6
Carteria species	A-6	25	.002	-	2	14	-	20	-
"	A-6	25	.008	4	4	2	10	10	5
Platymonas subcordiformis	A-6	25	.003	-	5	3	-	33	20
Chlamydomonas species	B-2	50	.002	3	2	2	30	20	20
Nannochloris atomus	B-2	50	.005	2	-	2	8	-	8
P-141[2]	B-6	50	.018	2	2	-	2	2	-
Stichococcus basillaris[1]	B-6	50	.003	6	-	-	40	-	-
Chlorella species	B-6	50	.009	3	8	2	7	17	4
"	B-6	50	.019	3	-	-	3	-	-
Carteria chuii	B-6	50	.002	6	-	5	60	-	50
"	B-6	50	.008	7	8	7	17	20	17

[1] Normally considered to be fresh water variety, but obtained here from marine culture.

[2] Unidentified.

* Reported only where uptake took place.

TABLE 5 (continued)

PROJECTED UPTAKES BASED ON EXPERIMENTAL RESULTS

Marine Algae

Species	Medium		Final Density PCV (ml/ml)	Measured uptake from medium (as % of original medium)*			Calculated uptake from medium based on assumed pcv of .02 ml/ml (as % of original medium)*		
	Code	% Sea Water		Na^+	Cl^-	Cond.	Na^+	Cl^-	Cond.
Carteria species	B-6	50	.004	2	4	2	10	20	10
"	B-6	50	.012	3	3	4	5	5	6
Platymonas subcordiformis	B-6	50	.004	-	2	-	-	10	-
"	B-6	50	.011	3	5	3	5	9	5
Dunaliella salina	B-6	50	.003	10	10	-	67	67	-
Dunaliella euchlora	C	100	.025	3	5	5	2	4	4
Monochrysis	C	100	.002	-	7	6	-	30	40
Dunaliella salina	C	100	.009	-	3	-	-	7	-
"	C	100	.020	-	4	3	-	4	3
Chlorella species (Lewin)	C	100	.015	-	4	4	-	5	5
Brachiomonas pulsifera	F	75	.005	2	3	4	8	12	16

* Reported only where uptake took place.

fairly constant. In reading these tables, it should be borne in mind that the values reported are the percentages of uptake from the original medium rather than the percentages remaining in the medium as reported in Tables 2 and 3.

VII Conclusions

Numerous algal species have been shown to concentrate sodium, chloride or both. This ability has been demonstrated in 25, 50, 75 and 100 percent sea water media. In many instances, the degree of concentration seems considerable. It has been pointed out, however, that the data obtained does not lend itself to detailed statistical analysis. The experimental results are sufficient to warrant further study of the uptake of sodium and chloride by algae. As a first step, experiments with some of the more promising algae reported herein should be repeated. Special care should be taken to preclude the introduction of possible sources of error discovered during the course of the recent work. Should the repeat experiments verify the original results, algae will have assumed a significant role in saline water conversion research. The scope of the research should then be broadened to study additional species of algae and sodium and chloride secretion tests should be tried on those species showing good uptake. In addition, the kinetics of uptake and secretion should be studied. The uptake data reported herein do not indicate the rate of uptake. This might best be obtained by growing dense fresh water cultures of those algae that can be grown in fresh water, removing the algae and then placing them in saline water. Uptake rate determinations could be made by removing aliquots at periodic intervals.

Another result of this work is worthy of comment. It was quite surprising to find that many species of fresh water algae grew very well in 25-and 50-percent sea water medium. Little work of this nature had previously been performed. The literature is similarly scant with respect to the culture of marine algae. A number of the marine algae responded very well to the culture conditions. They achieved considerable densities in a few days and, at times, doubled their populations within 24-hour periods. It may, therefore, be that fresh water algae grown in saline water or marine algae directly could be mass-cultured for protein production. This could be of much potential interest to those arid areas bordering sea water, such as the mideast countries. The scarcity of food and fresh water go hand-in-hand in these lands. It might be possible to grow quantities of algae for use as cattle fodder, or perhaps at some future date food for humans, without consuming fresh water. This, in effect, would achieve one of the goals of the Office of Saline Water which is to convert sea water for use in the production of crops. While the process would not convert the sea water, it would use it to produce a high-protein end-product. Should any processing of the algae be necessary to reduce the salt content, this could very likely be achieved using far less fresh water that would have been required to grow an equivalent crop. It would seem, therefore, that the study of salt-tolerable and marine algae for protein production might prove profitable.

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